



# Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide

Edition November 2003



Agilent Technologies

# Notices

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**Manual Part number:** G2946-90001

**Edition.** 11/2003

Printed in Germany

Agilent Technologies, Deutschland GmbH  
Hewlett-Packard-Strasse 8  
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A **WARNING** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a **WARNING** notice until the indicated conditions are fully understood and met.

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# Welcome

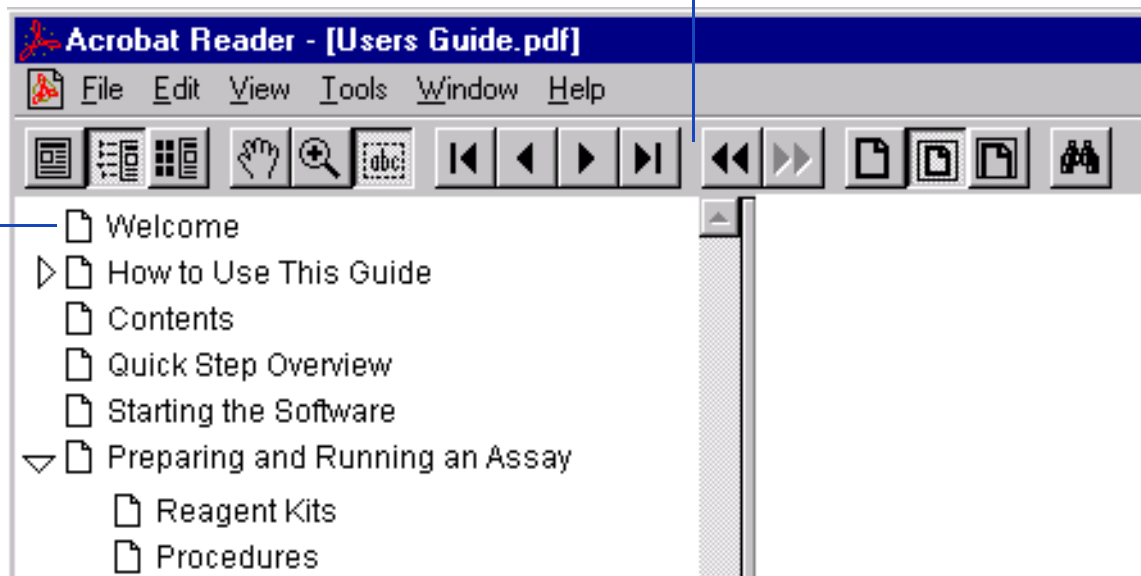
Welcome to the Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Guide. This online manual provides novice and advanced users with information needed to successfully run chips with the Agilent 2100 Bioanalyzer.

A quick look at “[How to Use This Guide](#)” on page 8 explains how easy it is to use this online manual and helps you to get started.

## How to Use This Guide

Use the interactive bookmarks in this frame to choose your desired topic.

Use Acrobat Reader's navigation bar to move around within a topic, see Navigating within Acrobat Reader



Click here to go to the table of contents

Here's the current page number

▲ displays previous page

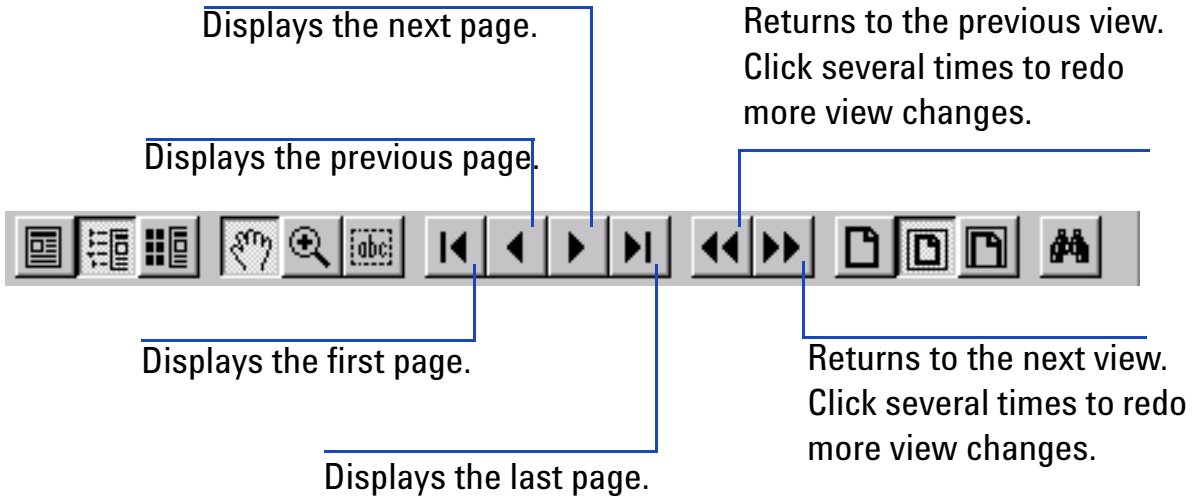
▼ displays next page

Click here to go to the index



# Navigating within Acrobat Reader

When you've chosen a topic with the bookmarks, use the buttons in Acrobat Reader's tool bar to move around within a topic.



For more information, see the Reader Online Guide in the Help menu.

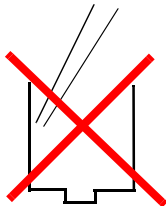
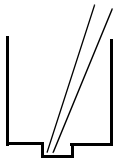
# Essential Measurement Practices

This section lists all user relevant hints on handling tools, chips, reagents and Agilent 2100 Bioanalyzer. For the latest information on assay-related hints, go to the Lab-on-a-Chip web site at:

<http://www.agilent.com/chem/labonachip>

## Tools and Handling

- Always follow the GLP-rules established in your laboratory.
- Always wear gloves when handling chips to prevent them from getting contaminated.
- When pipetting sample, use pipette tips that are small enough. Pipette tips that are too large will lead to poor quantitation accuracy.
- Change pipette tips between two pipetting steps to avoid cross-contamination.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well leads to bubbles and poor results. Holding the pipette at a slight angle will ensure proper dispensing of the liquid.



- Use a new syringe and electrode cleaner with each new LabChip Kit.
- For flow cytometric assays: Use inverse pipetting for chip preparation. When filling the pipette tip, push slightly over the first resistance. Empty the pipette tip only to the first resistance. This procedure avoids the introduction of bubbles and ensures pipetting the right volume.

## Chip Priming Station

- For the correct position of the syringe clip and base plate please refer to the appropriate Reagent Kit Guide.
- Replace the syringe with each new Kit.
- Check the performance of the chip priming station by applying the seal test on a monthly basis. For details see “[Maintenance of the Chip Priming Station](#)” on page 201. If necessary replace the gasket and/or adapter (reorder no. for gasket kit: G2938-68716).

## Reagents and Reagent Mixes—General

- Handle and store all reagents according to the instructions given in the specific Reagent Kit Guide.

- Keep all reagents and reagent mixes (for example, the gel-dye mixture) refrigerated at 4 °C when not in use for more than 1 hour. Reagents might decompose, leading to poor measurement results.
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes before use.

## **Gel and Gel-Dye**

- Use gel-dye mixture within four weeks of preparation. The gel-dye mixture might decompose and lead to poor measurement results.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light

## **Samples**

- Refer to the assay specific Reagent Kit Guides for maximum allowed sample and salt concentration.
- For RNA assays: Heat denature all RNA samples and RNA ladder for 2 min at 70 °C before use.
- For protein assays: Use 0.5 ml tubes for denaturation. Using larger tubes lead to poor results.

## Chips

- Prepared chips must be used within 5 minutes. Reagents might evaporate, leading to poor results.
- Vortex chips at the appropriate time of 1 minute. Inappropriate vortexing leads to poor results. Use only the IKA vortexer for chip vortexing. Replace the chip adapter (p/n 5022-2190) if it is wore out.
- Do not touch wells of the chip. The chip could get contaminated and this leads to poor measurement results.
- Do not leave any wells of the chip empty, or the assay will not run properly.

For DNA and RNA assays: Add 1  $\mu\text{l}$  of sample buffer to each unused sample well so that the total liquid volume in each well is at least 6  $\mu\text{l}$ .

For protein assays: pipette a sample or ladder replicate in any empty sample well.

For flow cytometry assays: Pipette 10  $\mu\text{l}$  of Cell Buffer or a sample replicate to each unused sample well so that the total liquid volume in each sample well is 10  $\mu\text{l}$ . If a well is kept empty or a different buffer is used, the pressure cartridge may get clogged due to bubble formation in the priming well.

- Do not touch the underside of the chip.

## Agilent 2100 Bioanalyzer

- Don't touch the Agilent 2100 bioanalyzer during a run and never place it on vibrating ground or near to air-circulating instruments (e.g. Temp-Cycters).

- Do not use force to press the chip in the receptacle of the Agilent 2100 bioanalyzer. The pressure or the electrode cartridge might get damaged when you close the lid. Check if the chip selector is in the required position.
- Cartridge cleaning:  
For electrophoresis assays: Clean electrodes on a daily basis using the electrode cleaner. For more details, see “[Maintenance of the Electrode Cartridge](#)” on page 180.  
For flow cytometry assays: If necessary, use a tissue to dry off any liquid at the pressure adapter at the end of a run.
- Clean electrodes on a quarterly basis using a toothbrush and distilled water. For more details, see “[How to clean the Pin Set of the Electrode Cartridge](#)” on page 192.
- Clean the focusing lens once a month (or after any liquid spill) using isopropanol see “[Cleaning the Lens](#)” on page 222.

# Troubleshooting the Instrument Communication

To check whether your PC communicates with the Agilent 2100 bioanalyzer:

- 1** Start the instrument. The power switch is located at the rear where the power cable plugs in.  
The status indicator lamp will light green if power is present and all instrument self-tests have been passed successfully.
- 2** Start the 2100 expert software and select the instrument tab in the *Instrument context*. In the tree view, highlight the appropriate instrument.

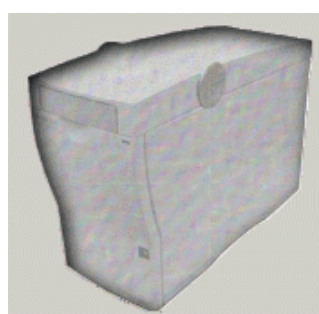
- 3 Open and close the lid—the icon in the instrument context should change from closed to open, see Figure 1, “2100 bioanalyzer icons showing the instrument status”



bioanalyzer online  
and lid closed.



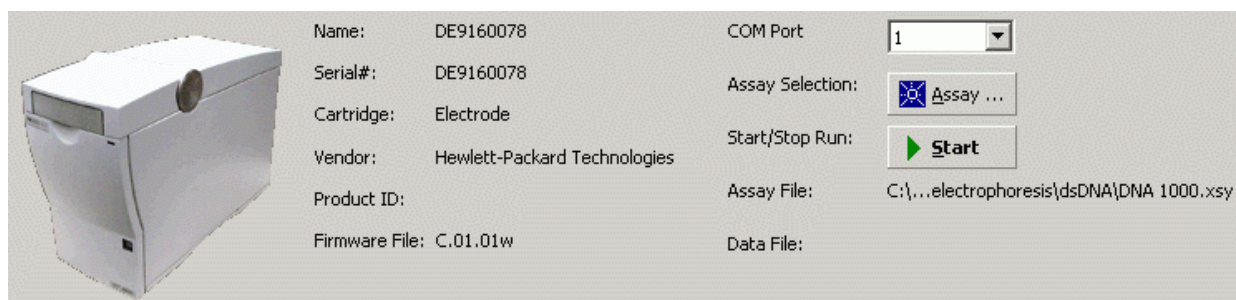
bioanalyzer online  
and lid open.



Dimmed icon: instrument  
switched off or not connected  
to PC.

**Figure 1 2100 bioanalyzer icons showing the instrument status**

If the instrument is connected successfully, additional hardware information (serial number, cartridge type,...) is displayed on top of the screen, see Figure 2, “Bioanalyzer hardware information”.



**Figure 2 Bioanalyzer hardware information**



If the icon doesn't change:

- Check the M-port settings in the 2100 expert software, see "[Changing COM-Port Settings](#)" on page 17.
- Check whether the status indicator is red. If it is red, turn off line power to the Agilent 2100 bioanalyzer and turn on again. If the problem persists, contact Agilent Technologies.
- Check whether the status indicator is on. If it is off, replace the fuses as described under "[Changing the Fuses of the Agilent 2100 Bioanalyzer](#)" on page 223. A set of spare fuses comes with the instrument.
- Check that the RS232 communication cable is connected as described in the Installation and Safety Guide.
- Check if another hardware device is connected to your computer via RS232 cable.
- Replace the RS232 cable.
- Reinstall the Agilent 2100 bioanalyzer software.
- If the Agilent 2100 bioanalyzer will still not communicate, contact Agilent Technologies.

## Changing COM-Port Settings

The Agilent 2100 bioanalyzer communicates via a serial RS232 cable with your PC. The number of COM-ports available, depends on the type of your PC. Laptop PCs have only one COM-Port. The standard desktop PC that is shipped with the Agilent 2100 bioanalyzer contains two COM-ports. The bioanalyzer software allows to adjust the COM-port.

To change the COM-Port settings:

- 1 Select the *Instrument* tab in the *Instrument context*. In the tree view, highlight the appropriate instrument.
- 2 Under *COM Port* choose a different port number from the drop down list.
- 3 Check the icon of the Agilent 2100 bioanalyzer on the screen. If it is no longer dimmed, communication between the Agilent 2100 bioanalyzer and PC is working properly. In addition, hardware information is displayed, see Figure 2, "[Bioanalyzer hardware information](#)" on page 16.
- 4 If you have a PC connected to your instrument and the icon is still dimmed, repeat step 2, choosing a different COM Port each time, until it is not dimmed anymore. If the Agilent 2100 bioanalyzer will still not communicate, contact Agilent Technologies.

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#### NOTE

The demo-port refers to demo assays that do not require a PC-instrument communication. For more information on demo assays please refer to the Online Help or User's Guide.

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## The Multi COM-Port Card

A Multi Port Card (e.g. RocketPort PCI/Quad DB9) is installed in PCs that were shipped with the multi-instrument system (G2942AA). This system bundle was obsoleted in January 2004. The Agilent 2100 bioanalyzer is connected via a Multi Port Card/Multi Port

cable with the PC. Re-configuration of the Multi COM-Port Card might be necessary after PC-repair or re-installation of the operating system. This will enable multi-instrument support for the 2100 bioanalyzer again.

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#### NOTE

With the Multi COM-Port Card, not more than 2 instruments are supported with the 2100 expert software.

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## Re-configuration of the Multi-COM Port Card

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#### NOTE

After re-installation of the operating system, make sure that the MultiPort Cable (reorder number G2938-81610) is plug- in. Windows 2000 will automatically detect the MultiPort Card and install the necessary driver.

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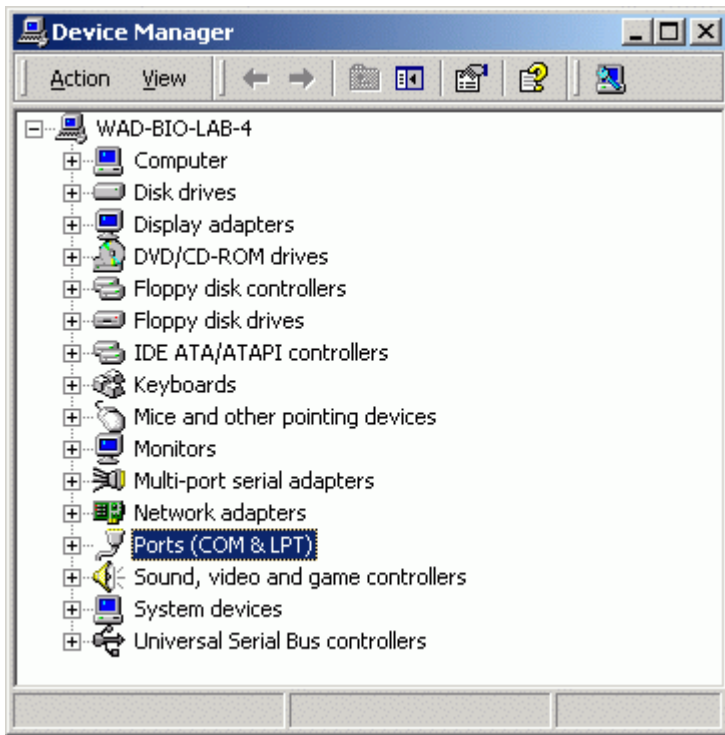
The setup process of the MultiPort Card requires 2 steps:

- 1 “Disabling the Standard PC Serial Ports (COM1 and COM2)” on page 19 .
- 2 “Assigning COM1 and COM2 to the RocketPort Card” on page 24.

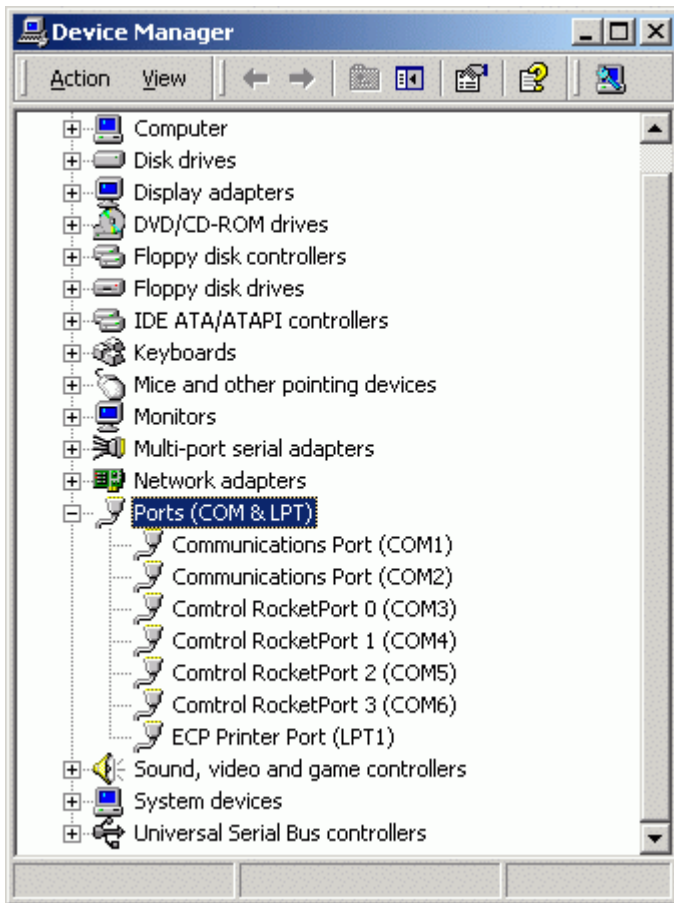
## Disabling the Standard PC Serial Ports (COM1 and COM2)

The embedded serial ports of your PC must be disabled before you can use your RocketPort card. To do so,

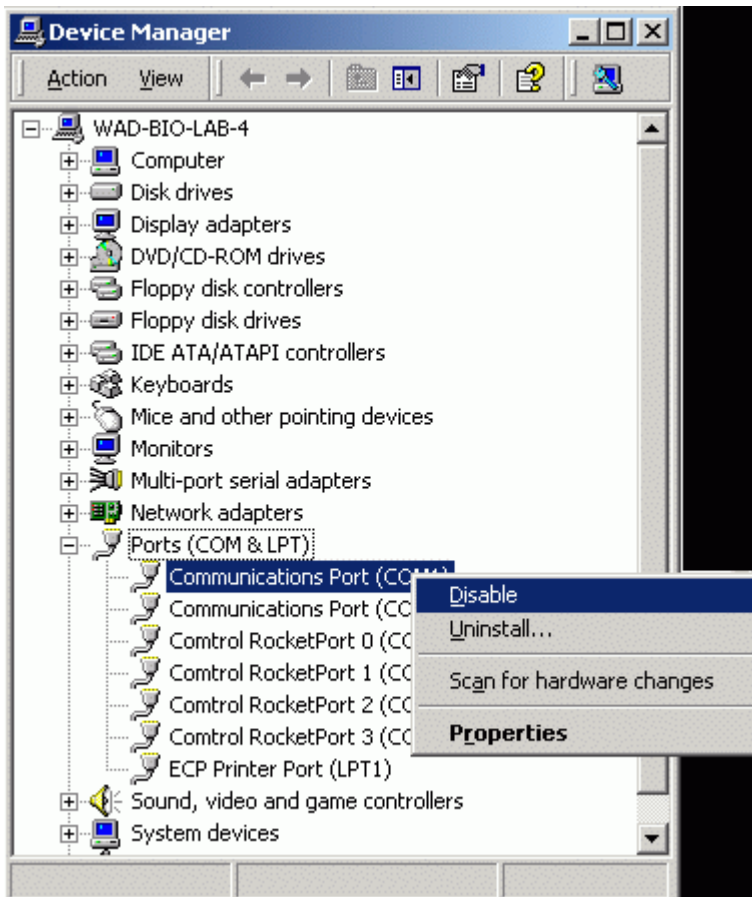
- 1 Select Start> Settings> Control Panel> System> Hardware> Device Manager:



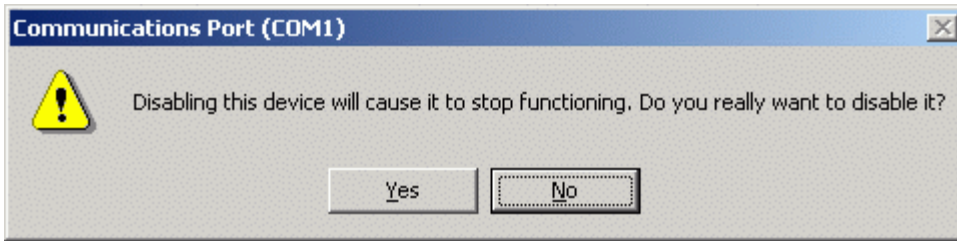
2 Double click the symbol called "Ports (COM & LPT)"



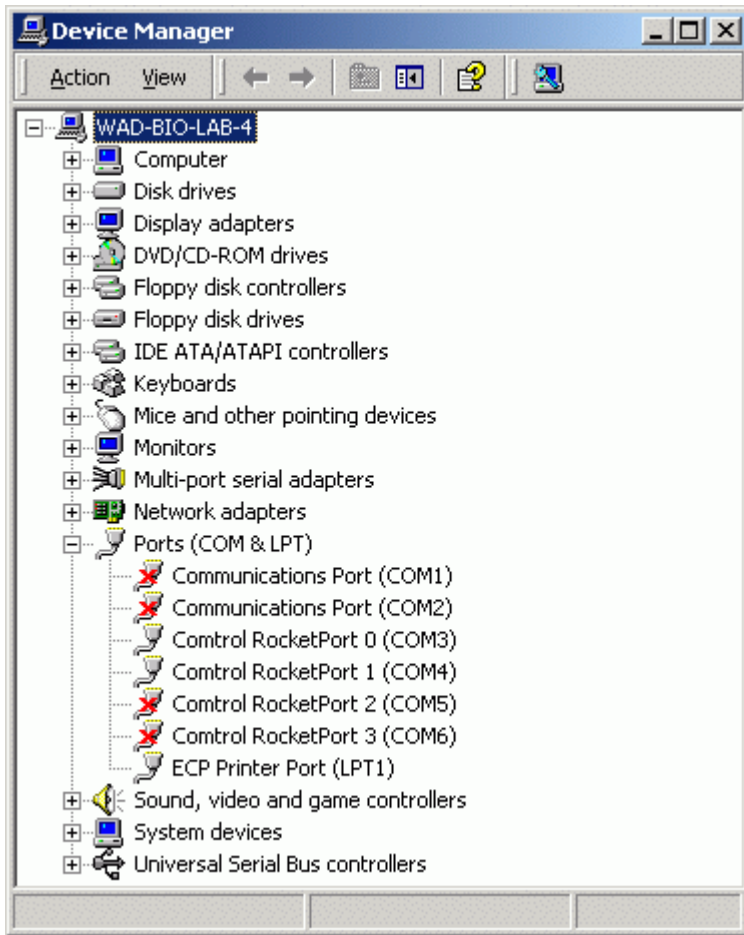
- 3 Highlight Communication Port (COM1) and disable this port by a right-mouse click and selecting 'Disable'.



4 In the upcoming dialog box confirm to disable the COM-port.



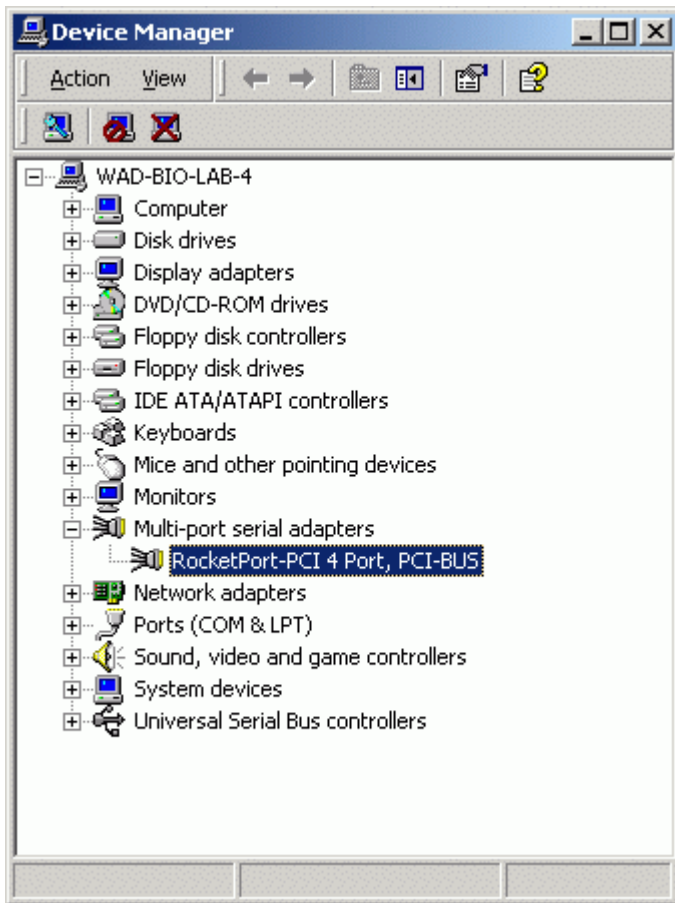
- 5 Repeat the previous steps to disable *Communication Port (COM2)*, *Communication Port 2 (COM5)* and *Communication Port 3 (COM6)*. Return to the Device Manager:



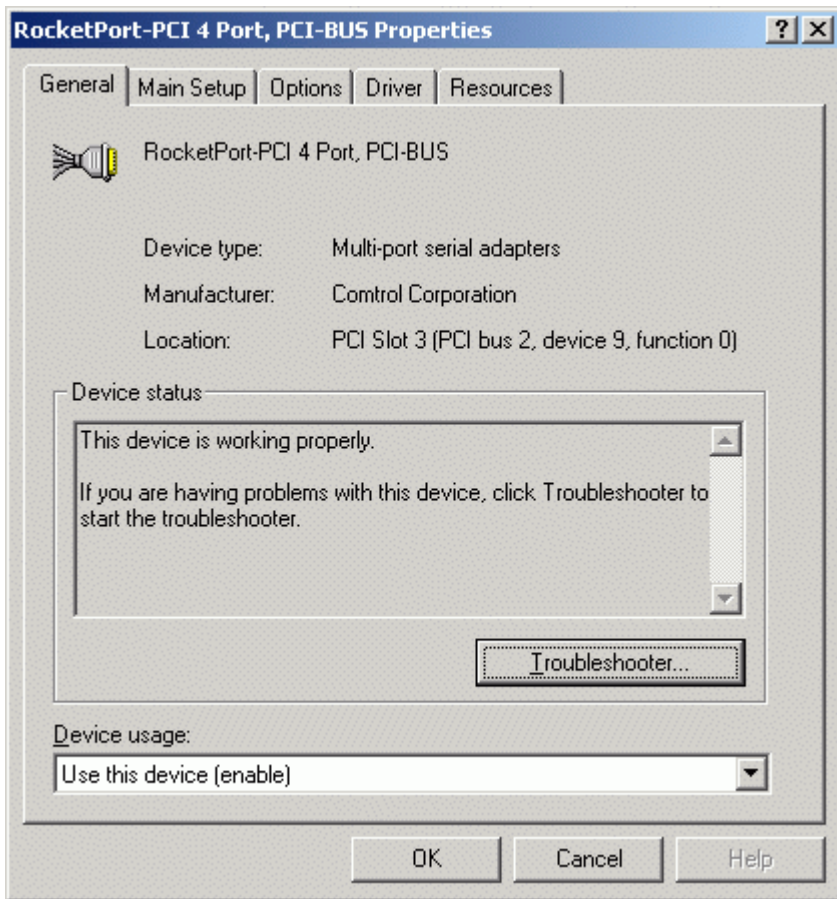
## Assigning COM1 and COM2 to the RocketPort Card

- 1 Select Start> Settings> Control Panel> System> Hardware> Device Manager. Double click the symbol called "Multi-port serial adapters":

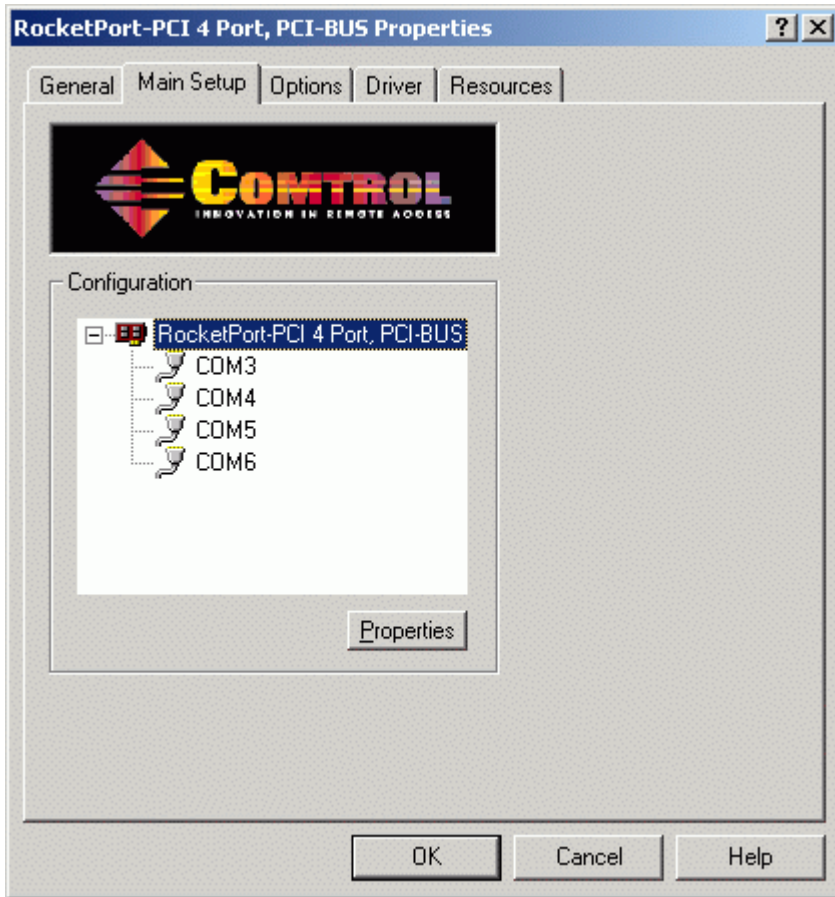




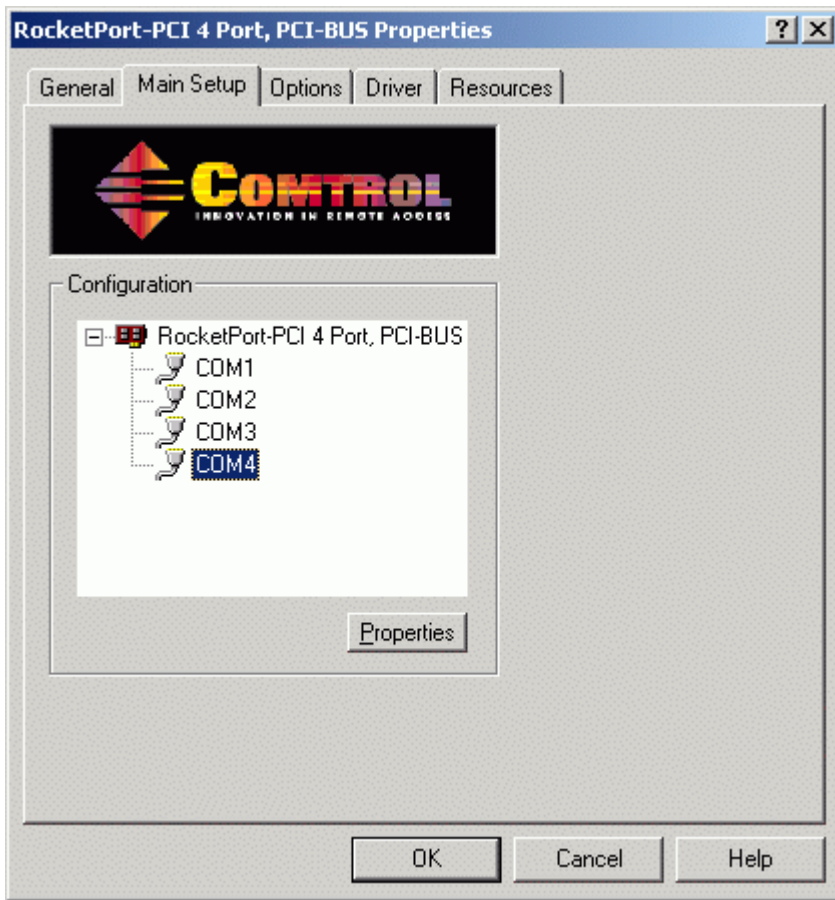
- 2 Double click the symbol called “RocketPort-PCI 4 Port, PCI-BUS” to open the RocketPort-PCI 4 Port, PCI-BUS Properties box:



### 3 Select "Main-Setup":



4 Alter the names from COMX to COM1....COM2:

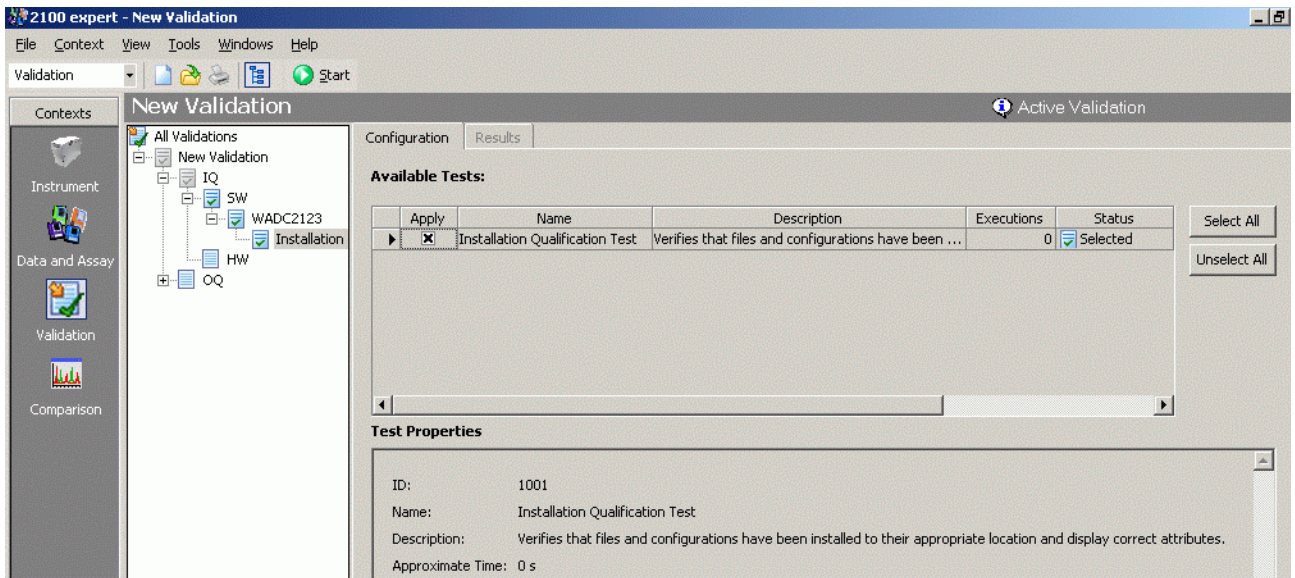


5 Select "OK" to confirm the changes.

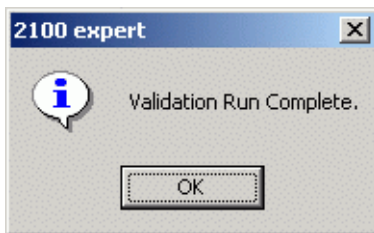
# Troubleshooting the 2100 Expert Software

If you suspect that the 2100 expert software is not working properly, you can check for corrupted or missing files.

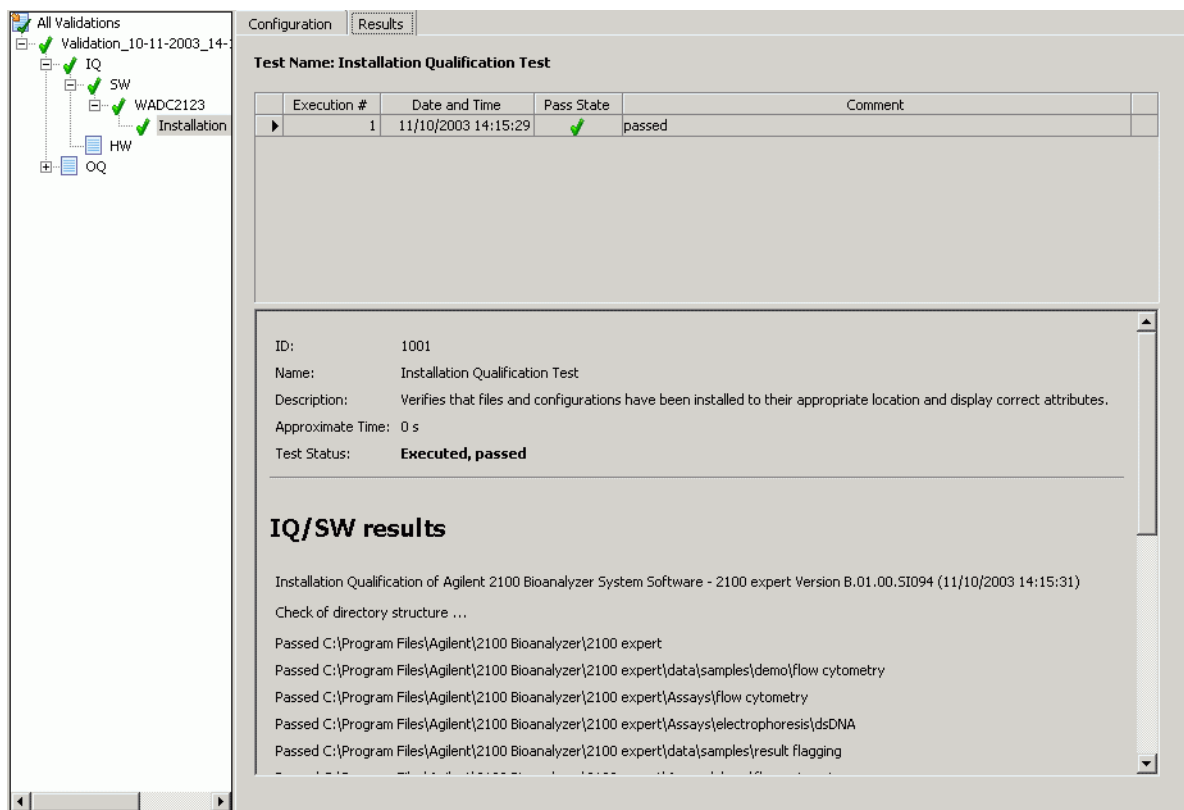
- 1 Start the 2100 expert software and select the validation context.
- 2 Select *File>New* to open a new validation.
- 3 In the tree view, select *New Validation>IQ>SW>my PC Name>Installation Qualification Test*.
- 4 Under *Available Tests* select the checkbox of the Installation Qualification Test. This test verifies that files and configurations have been installed to their appropriate location and display correct attributes.



- 5 Start the software test tool by clicking the *Start* button.
- 6 The *Save As* dialog box appears. Define the name and location of the validation file.
- 7 When the test is finished, the *Validation Run Complete* message appears.



- 8 The result of the installation qualification test depends on whether the software installation is complete and no files are corrupted. To review the results, switch to the *Results* tab:



The screenshot displays the 'Results' tab of a software interface. On the left, a tree view shows a hierarchy of validation items: 'All Validations' (expanded), 'Validation\_10-11-2003\_14-' (expanded), 'IQ' (expanded), 'SW' (expanded), 'WADC2123' (expanded), 'Installation' (expanded), 'HW', and 'OQ'. The main area shows the 'Test Name: Installation Qualification Test'. Below this is a table with the following data:

Execution #	Date and Time	Pass State	Comment
1	11/10/2003 14:15:29	✓	passed

Below the table, the test details are shown:

ID: 1001  
Name: Installation Qualification Test  
Description: Verifies that files and configurations have been installed to their appropriate location and display correct attributes.  
Approximate Time: 0 s  
Test Status: **Executed, passed**

**IQ/SW results**

Installation Qualification of Agilent 2100 Bioanalyzer System Software - 2100 expert Version B.01.00.SI094 (11/10/2003 14:15:31)

Check of directory structure ...

- Passed C:\Program Files\Agilent\2100 Bioanalyzer\2100 expert
- Passed C:\Program Files\Agilent\2100 Bioanalyzer\2100 expert\data\samples\demo\flow cytometry
- Passed C:\Program Files\Agilent\2100 Bioanalyzer\2100 expert\Assays\flow cytometry
- Passed C:\Program Files\Agilent\2100 Bioanalyzer\2100 expert\Assays\electrophoresis\dsDNA
- Passed C:\Program Files\Agilent\2100 Bioanalyzer\2100 expert\data\samples\result flagging

- 9 If the test passes and the Agilent 2100 bioanalyzer system still does not function correctly, see “[Troubleshooting the Instrument Communication](#)” on page 15 and “[Hardware Diagnostics](#)” on page 33 for further troubleshooting procedures. Finally, check your application, see

[“Troubleshooting the DNA Application”](#) on page 39,  
[“Troubleshooting the RNA Application”](#) on page 80,  
[“Troubleshooting the Protein Application”](#) on page 119 or  
[“Troubleshooting the Cell Application”](#) on page 166.

- 10** If the test fails, reinstall the 2100 expert software using the software CD-ROM that is supplied with the system. Follow the instructions that are printed on the CD-ROM.
- 11** If the test continues to fail, contact Agilent Technologies.



# Hardware Diagnostics

Several tests are provided for the Agilent 2100 bioanalyzer to check the functionality of the hardware. You should perform the tests on a regular basis, or if incorrect measurements or error messages occur. Depending on the assay type (electrophoresis assays or flow cytometry assays), a different set of tests is available and different test chips have to be used. Test chip bundles are part of the bioanalyzer electrophoresis set (G2947CA) and flow cytometry set (G2948CA). Table 1, “[Different Test Chips](#)” provides an overview.

**Table 1 Different Test Chips**

Test Chip Type	Comment	quant.
Test Chip Kit for Electrophoresis Assays (reorder no. G2938-68100)		
Autofocus Test Chip	Values for fluorescence and offset are printed on the chip; can be used multiple times	1
Electrode/Diode Test Chip	can be used multiple times	1
Leak Current Test Chip	has to be prepared with deionized water, can only be used once	5

Test Chip Type	Comment	quant.
Test Chip Kit for Flow Cytometry Assays (reorder no. G2938-68200)		
Cell Autofocus Test Chip	required for multiple tests, can be used multiple times	1

Table 2, “[Diagnostic tests available for electrophoresis set-up](#)” shows a complete list of hardware diagnostic tests that can be run with the electrode cartridge.

**Table 2 Diagnostic tests available for electrophoresis set-up**

Test	Description
Electronic test	Verifies proper functioning of all electronic boards.
Fan test	Checks that the fan is running.
Lid sensor test	Checks for the devices sensing for open or closed lid, and for laser and LED off when lid is being closed.
Temperature test	Checks that the temperature ramp up speed of the heater plate is within specifications.
Stepper motor test	Checks for proper movement of the stepper motor.
Electrode/diode test	Checks photodiode and current versus voltage performance of the 2100 bioanalyzer. Electrode/diode test chip required.
High voltage stability test	Checks the accuracy and stability of all 16 high voltage power supplies. Unused chip (DNA, RNA or protein) required.

Test	Description
High voltage accuracy test	Check of the high voltage controller.
High voltage accuracy-on load test	Check of channel-reference diode in transmission direction.
Short circuit test	Checks for instrument leak currents using an empty chip. Note: the limits of this test specify an ambient temperature of 25°C and relative humidity less than or equal to 60%. Higher temperatures and relative humidity could result in a leak current.
Current leakage test	Measures electrode cartridge leak current(s) between pins. Leak current test chip required. For correct preparation of the leak current test chip, please refer to the technical note included in the test chip bundle.
Optics test	Checks for proper alignment of internal optics and proper function of the laser and LED.
Autofocus test	Checks focusing capability of optical system. Autofocus test chip required.
Laser stability test	Measurement of stability of red laser signal.

When running the 2100 bioanalyzer in flow cytometry set-up, the following hardware diagnostic tests can be run:

**Table 3 Diagnostic tests available for flow cytometry set-up**

Test	Description
Pressure offset test	Calibrates the pressure sensors to zero.
System leak test	First a pressure of -100 mbar is produced. Then, the pressure is monitored for changes. Cell Autofocus test chip required.
Pressure control test	Checks whether the Agilent 2100 bioanalyzer can hold the working pressure of -140 mbar. Cell Autofocus test chip required.
Cell autofocus test	Checks the focusing capability of the system. Cell Autofocus test chip required.

## Test Procedure

### NOTE

Diagnostic tests cannot be run while the 2100 expert software is performing a chip run.

For details on the test procedure, please refer to the documentation that comes with the test chip kits.

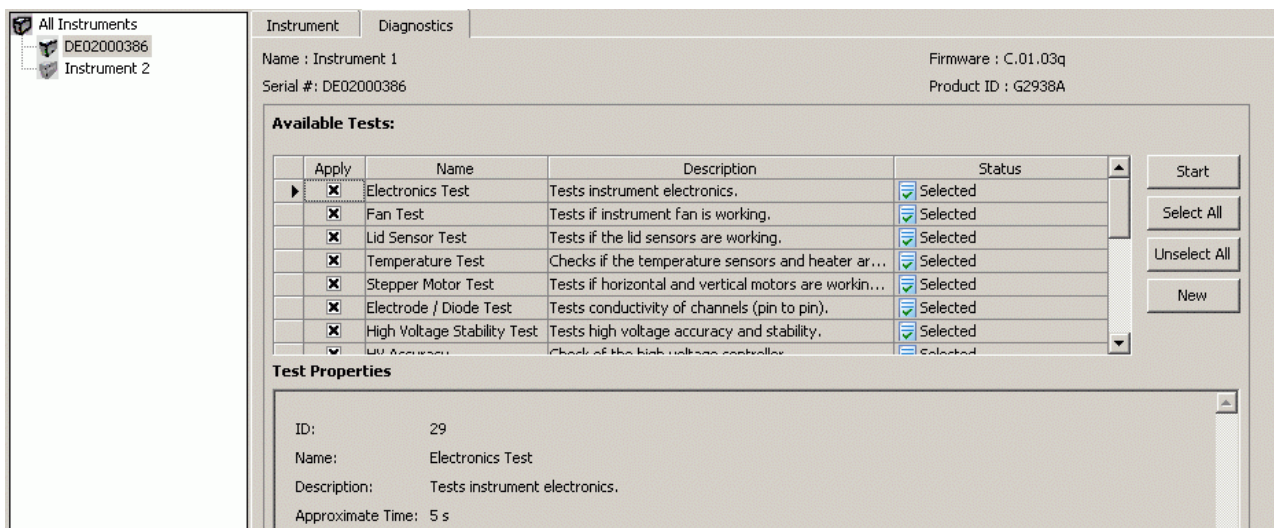
- 1 Start the 2100 expert software.
- 2 Access the hardware diagnostic tests by selecting the *Diagnostics* tab in the *instrument* context of the 2100 expert software.

- 3 In case more than one bioanalyzer is connected to you PC, highlight the appropriate instrument in the tree view.

## NOTE

You can only perform tests if the instrument is online. In offline mode the test entries are dimmed.

- 4 Select any of the hardware tests you want to apply from the list given or choose *Select All* to run all tests.



The screenshot shows the 'Diagnostics' tab in the software. The instrument details are: Name: Instrument 1, Serial #: DE02000386, Firmware: C.01.03q, and Product ID: G2938A. The 'Available Tests' table is as follows:

Apply	Name	Description	Status
<input checked="" type="checkbox"/>	Electronics Test	Tests instrument electronics.	<input checked="" type="checkbox"/> Selected
<input checked="" type="checkbox"/>	Fan Test	Tests if instrument fan is working.	<input checked="" type="checkbox"/> Selected
<input checked="" type="checkbox"/>	Lid Sensor Test	Tests if the lid sensors are working.	<input checked="" type="checkbox"/> Selected
<input checked="" type="checkbox"/>	Temperature Test	Checks if the temperature sensors and heater ar...	<input checked="" type="checkbox"/> Selected
<input checked="" type="checkbox"/>	Stepper Motor Test	Tests if horizontal and vertical motors are workin...	<input checked="" type="checkbox"/> Selected
<input checked="" type="checkbox"/>	Electrode / Diode Test	Tests conductivity of channels (pin to pin).	<input checked="" type="checkbox"/> Selected
<input checked="" type="checkbox"/>	High Voltage Stability Test	Tests high voltage accuracy and stability.	<input checked="" type="checkbox"/> Selected
<input checked="" type="checkbox"/>	UV Accuracy	Check of the high voltage controller	<input checked="" type="checkbox"/> Selected

Below the table, the 'Test Properties' section shows:

ID: 29  
Name: Electronics Test  
Description: Tests instrument electronics.  
Approximate Time: 5 s

Buttons on the right include Start, Select All, Unselect All, and New.



- 5 Select *Start* and follow the instructions as given by the 2100 expert software.
- 6 At the end of the procedure all tests must be passed.
- 7 If the tests are not passed, redo the tests.

**8** If failures still persist, contact Agilent Technologies.

The results of diagnostic tests are stored in .xdy files in the 2100 exoert installation folder '...\diagnosis'. If tests fail, send the .xdy files to the Agilent service.

# Troubleshooting the DNA Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the Run Log for the data file. Select the Log Book tab in the Data and Assay context. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see “[Symptoms \(DNA\)](#)” on page 41.

For most observations you will find at least one corresponding example, depicting a typical electropherogram or result table. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by priority.

The causes are grouped into three levels:

- most probable cause
- probable cause

- least probable cause











A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by probability.



## Symptoms (DNA)

Click the icon to see an example, or go straight to the troubleshooting hints.

- [“Residual Gel in Spin Filter after Centrifugation”](#) on page 43
- [“Too High Quantitation Results”](#) on page 44
- [“Too Low Quantitation Results”](#) on page 45
- [“Wrong Sizing Result”](#) on page 46
- [“Poor Chip Performance Error Message”](#) on page 48
- [“Chip Not Detected”](#) on page 50

-  [“Additional Sample or Ladder Peaks”](#) on page 51
-  [“Spikes”](#) on page 54
-  [“Low Sensitivity”](#) on page 57
-  [“Missing Peaks”](#) on page 60
-  [“Missing Upper Marker”](#) on page 63
-  [“Broad Peaks”](#) on page 65
-  [“Baseline Dips”](#) on page 67
-  [“Baseline Noise”](#) on page 69
-  [“Baseline Jumps”](#) on page 71
-  [“Wavy Baseline”](#) on page 73
-  [“Late Migration”](#) on page 76

-  “Peak Tailing” on page 79

## Residual Gel in Spin Filter after Centrifugation

<b>Most Probable Causes</b>	<b>Solution</b>
Gel-dye mix was centrifuged at too low g-value.	Refer to the DNA Reagent Kit Guide for proper centrifuge settings.
Cooled centrifuge was used for preparation of gel-dye mix.	Repeat centrifugation step without cooling.
<b>Probable Causes</b>	<b>Solution</b>
Particles in the gel-dye mix.	Repeat the preparation of the gel-dye mix. Wear powder-free gloves only.

Back to [“Symptoms \(DNA\)”](#) on page 41

## Too High Quantitation Results

<b>Most Probable Causes</b>	<b>Solution</b>
Pipetting error during preparation of mixtures.	Check dilution procedure and check calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the speed to the set-point.
<b>Probable Causes</b>	<b>Solution</b>
Dye concentration too low (marker disappears).	Use dye concentration according to the DNA Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect dye from light during this time.
<b>Least Probable Causes</b>	<b>Solution</b>
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.

Back to [“Symptoms \(DNA\)”](#) on page 41

## Too Low Quantitation Results

<b>Most Probable Causes</b>	<b>Solution</b>
Pipetting error during preparation of mixtures.	Check dilution procedure and calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the speed to the set-point.
<b>Probable Causes</b>	<b>Solution</b>
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Sample concentration too high.	Use sample concentration according to the DNA Reagent Kit Guide.
<b>Least Probable Causes</b>	<b>Solution</b>
Dye concentration too high.	Use dye concentration according to the DNA Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix.

Back to [“Symptoms \(DNA\)”](#) on page 41

## Wrong Sizing Result

Most Probable Causes	Solution
DNA ladder degraded.	Check expiration date of reagents.
Loaded chip kept for too long before run.	Prepared chips must be used within 5 min.
Chip not properly primed. Clogged chip priming station or wrong settings of priming station	Use a new chip. Check chip priming station as described in “ <a href="#">Checking the Chip Priming Station for Proper Performance - Seal Test</a> ” on page 209. Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Chip contaminated.	Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
No ladder in ladder well.	Use a new chip.

<b>Least Probable Causes</b>	<b>Solution</b>
Vibration of Agilent 2100 bioanalyzer.	Don't touch Agilent 2100 bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 bioanalyzer in thermally stable environment.
High voltage power supply defective.	Check high voltage power supply using the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If the power supply is defective, contact Agilent Technologies.

Back to “[Symptoms \(DNA\)](#)” on page 41

## Poor Chip Performance Error Message

Most Probable Causes	Solution
Amount of liquid pipetted is too low or one or more wells of the chip are empty.	Check Reagent Kit Guide on amount of liquid to be pipetted. Check calibration of pipette.
Chip not properly primed. Clogged chip priming station or wrong settings of priming station.	Use a new chip. Check chip priming station as described in " <a href="#">Checking the Chip Priming Station for Proper Performance - Seal Test</a> " on page 209. Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
Probable Causes	Solution
Chip preparation with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes before use.
Chips were stored in the fridge/freezer.	Prepare a new chip. Store chips at room temperature.
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the " <a href="#">Hardware Diagnostics</a> " on page 33. If the power supply is defective, contact Agilent Technologies.



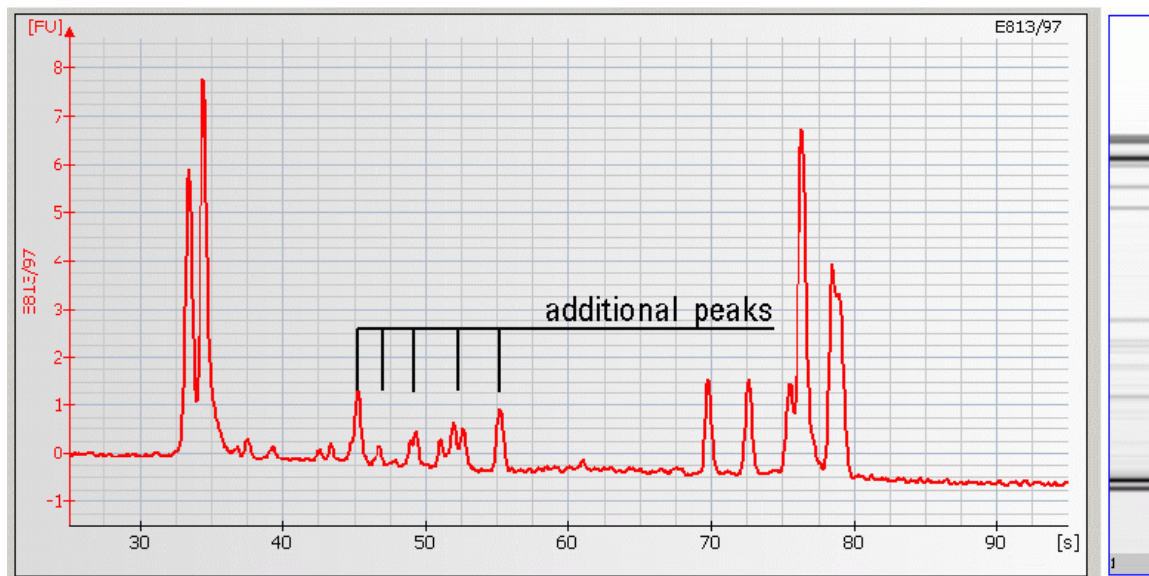
Back to [“Symptoms \(DNA\)”](#) on page 41

## Chip Not Detected

<b>Most Probable Causes</b>	<b>Solution</b>
Amount of liquid pipetted is too low or chip is empty.	Check assay procedure on amount of liquid to be pipetted. Pipette sample or buffer in all wells.
Chip not properly primed. Clogged chip priming station or wrong settings of priming station.	Use a new chip. Check chip priming station as described in <a href="#">“Checking the Chip Priming Station for Proper Performance - Seal Test”</a> on page 209. Check if clip and base plate of priming station are in the right position (see DNA Reagent Kit Guide).
<b>Probable Causes</b>	<b>Solution</b>
No communication between Agilent 2100 bioanalyzer and PC.	Check instrument communication as described in <a href="#">“Troubleshooting the Instrument Communication”</a> on page 15.
<b>Least Probable Causes</b>	<b>Solution</b>
High voltage power supply defective.	Check high voltage power supply using the <a href="#">“Hardware Diagnostics”</a> on page 33. If the power supply is defective, contact Agilent Technologies.

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## Additional Sample or Ladder Peaks



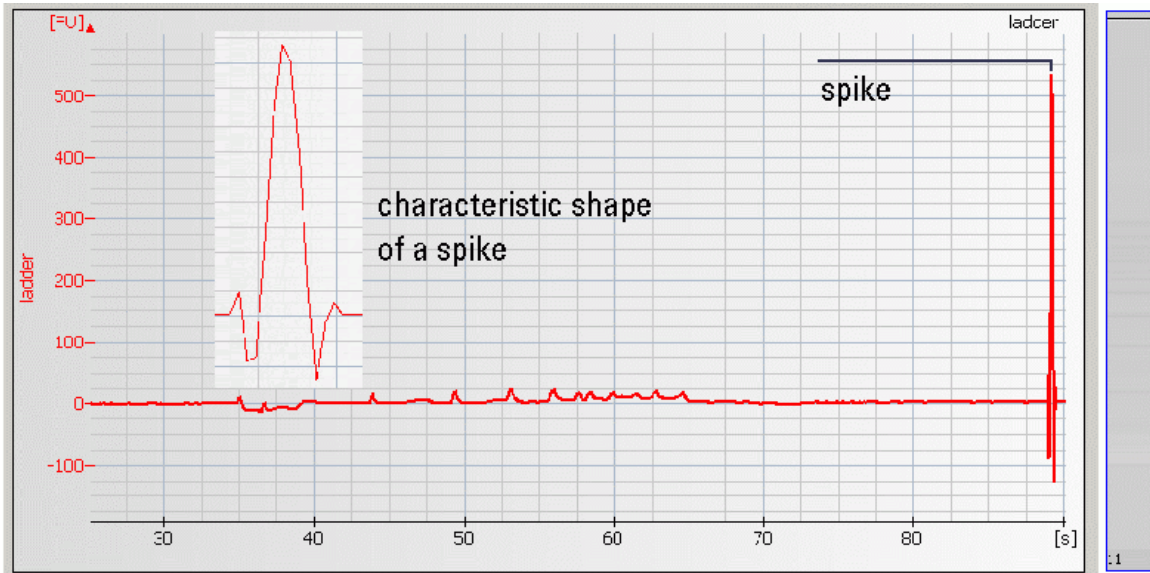
## Causes for Additional Sample or Ladder Peaks

<b>Most Probable Causes</b>	<b>Solution</b>
Chip contaminated.	Wear powder-free gloves only.
Dust particles in separation channels.	Clean the electrodes.  Load the chip immediately after taking it out of its sealed bag.
<b>Probable Causes</b>	<b>Solution</b>
Sample degraded or contaminated.	Always wear gloves when handling chips and samples to prevent them from getting contaminated.
Chip preparation with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes before use.
Chip not properly primed. Clogged chip priming station or wrong settings of priming station.	Use a new chip. Check chip priming station as described in " <a href="#">Checking the Chip Priming Station for Proper Performance - Seal Test</a> " on page 209). Check if clip and base plate of priming station are in the correct position (see DNA Reagent Kit Guide).
Vibration of Agilent 2100 bioanalyzer.	Don't touch Agilent 2100 bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.

Least Probable Causes	Solution
DNA ladder degraded.	Check expiration date of reagents.

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# Spikes



## Causes for Spikes

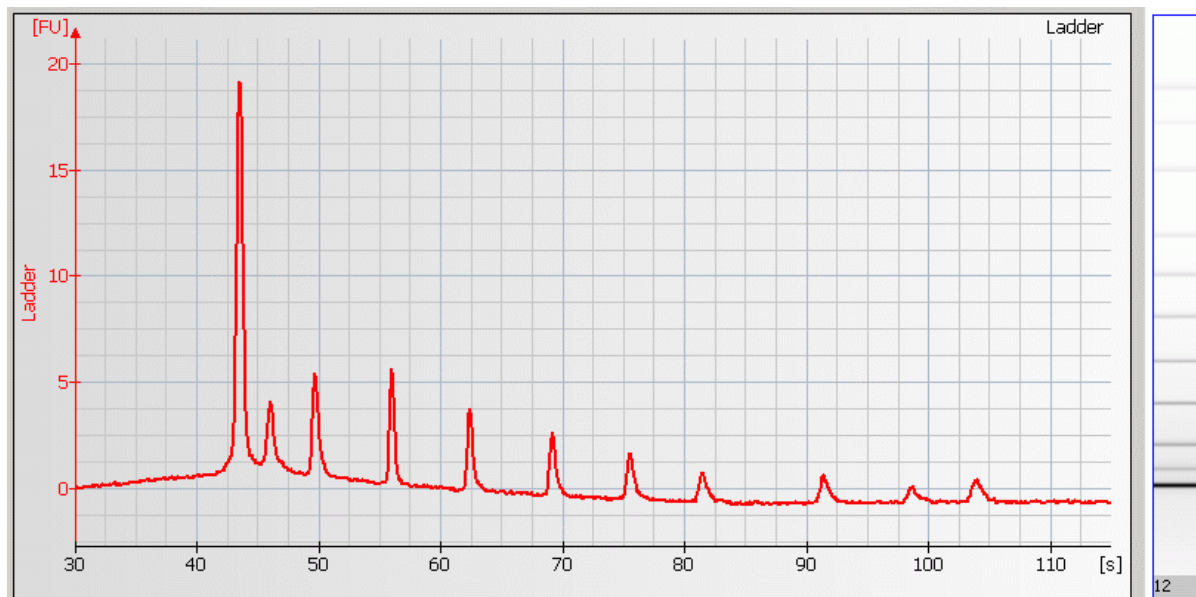
Most Probable Causes	Solution
Vibration of Agilent 2100 bioanalyzer.	Don't touch Agilent 2100 bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Chip/gel-dye mix contaminated with particles.	Prepare new chip with new gel-dye mix: Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Dye agglomerates present in the gel-dye mix.	Use dye concentration according to the Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.

<b>Probable Causes</b>	<b>Solution</b>
Chip preparation with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes before use.
<b>Least Probable Causes</b>	<b>Solution</b>
Power outlett	Install power filter.

Back to [“Symptoms \(DNA\)”](#) on page 41



## Low Sensitivity



12

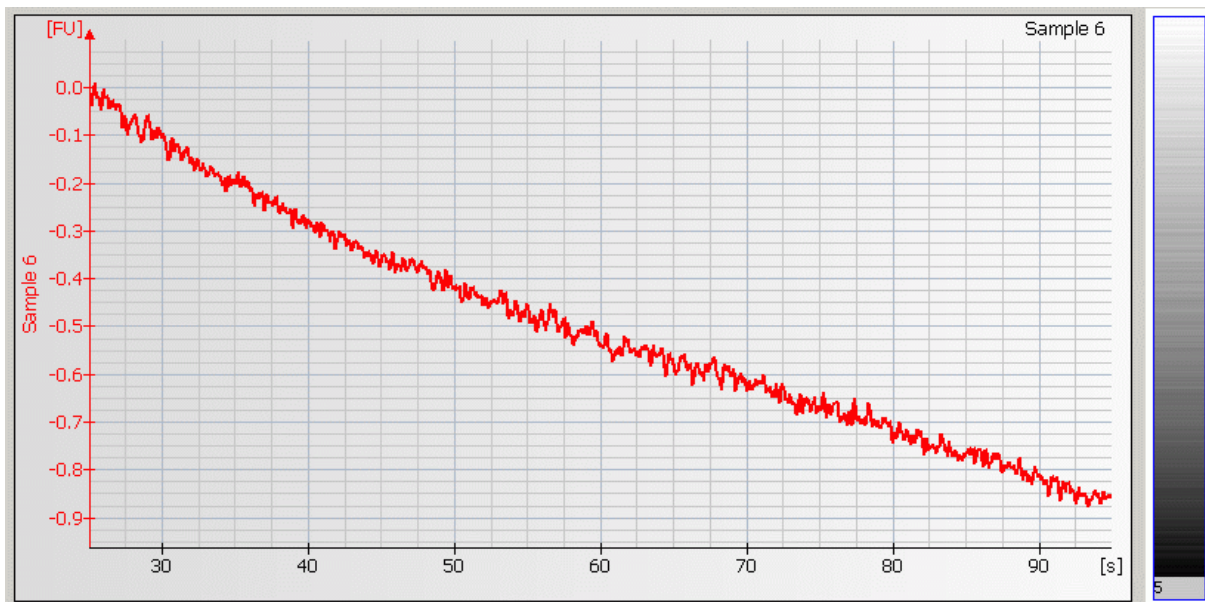
## Causes for Low Sensitivity

Most Probable Causes	Solution
Dye concentration too low.	Use dye concentration according to the Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.
Pipetting error during preparation of mixtures.	Check dilution procedure and calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Holding the pipette at a slight angle will ensure proper dispensing of the liquid. Use appropriate pipette and tips.
Probable Causes	Solution
Fingerprint on focusing lens or on the backside of the chip.	Clean lens as described in <a href="#">“Cleaning the Lens”</a> on page 222.  Do not touch the underside of the chip.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use IKA shaker for chip vortexing. Adjust speed to set-point.

Least Probable Causes	Solution
Chip contaminated.	<p>Wear powder-free gloves only.</p> <p>Don't touch the underside of the chip.</p> <p>Don't touch the wells of the chip.</p> <p>Clean the electrodes.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
Vibration of Agilent 2100 bioanalyzer.	<p>Don't touch Agilent 2100 bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.</p>
Autofocus failure.	<p>Check autofocus using the “<a href="#">Hardware Diagnostics</a>” on page 33. If autofocus fails, contact Agilent Technologies.</p>

Back to “[Symptoms \(DNA\)](#)” on page 41

# Missing Peaks



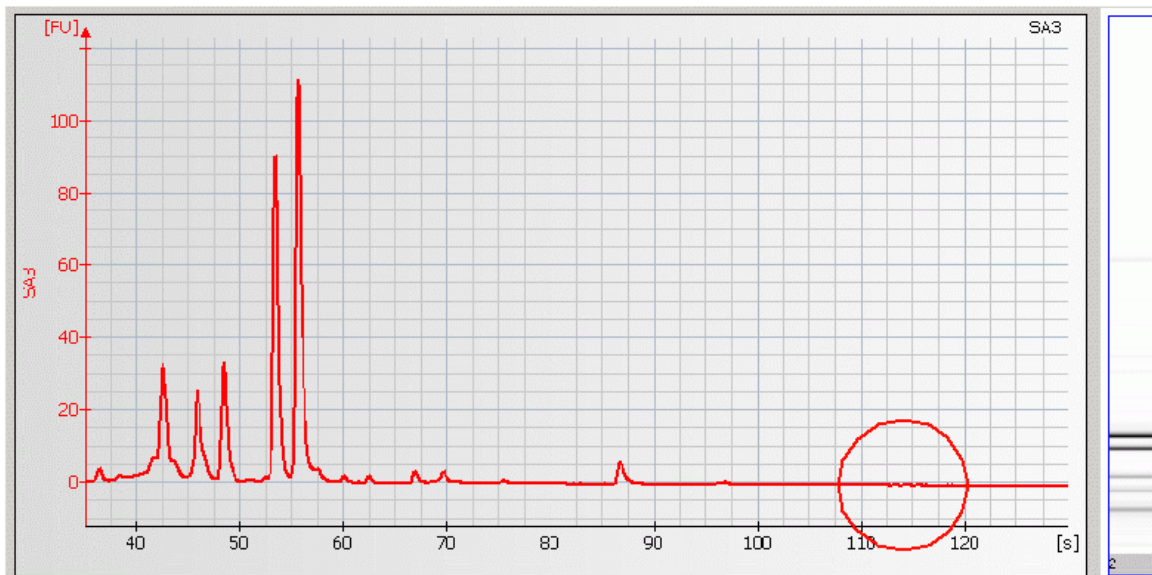
## Causes for Missing Peaks

Most Probable Causes	Solution
Too high salt concentration in sample	Refer to the maximum sample buffer salt concentration as specified in the reagent kit guide. Dilute samples with deionized DNase free water if necessary.
Clogged gasket and plastic adapter of priming station	Check chip priming station as described in <a href="#">“Checking the Chip Priming Station for Proper Performance - Seal Test”</a> on page 209). Clean/replace gasket and plastic adapter if necessary.
Wrong settings of the chip priming station.	Check if clip and base plate of priming station are in the right position (see Reagent Kit Guide).
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.

Probable Causes	Solution
Laser broken.	Perform Laser/LED/Optics test and Autofocus test as described in “ <a href="#">Hardware Diagnostics</a> ” on page 33. If tests fail contact Agilent Technologies.
Least Probable Causes	Solution
Autofocus failure or high voltage power supply defective	Check autofocus and high voltage power supply by means of the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If diagnostic fails, contact Agilent Technologies.
Sample wells filled with water.	Refer to the Reagent Kit Guide for proper preparation of the chip.

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# Missing Upper Marker



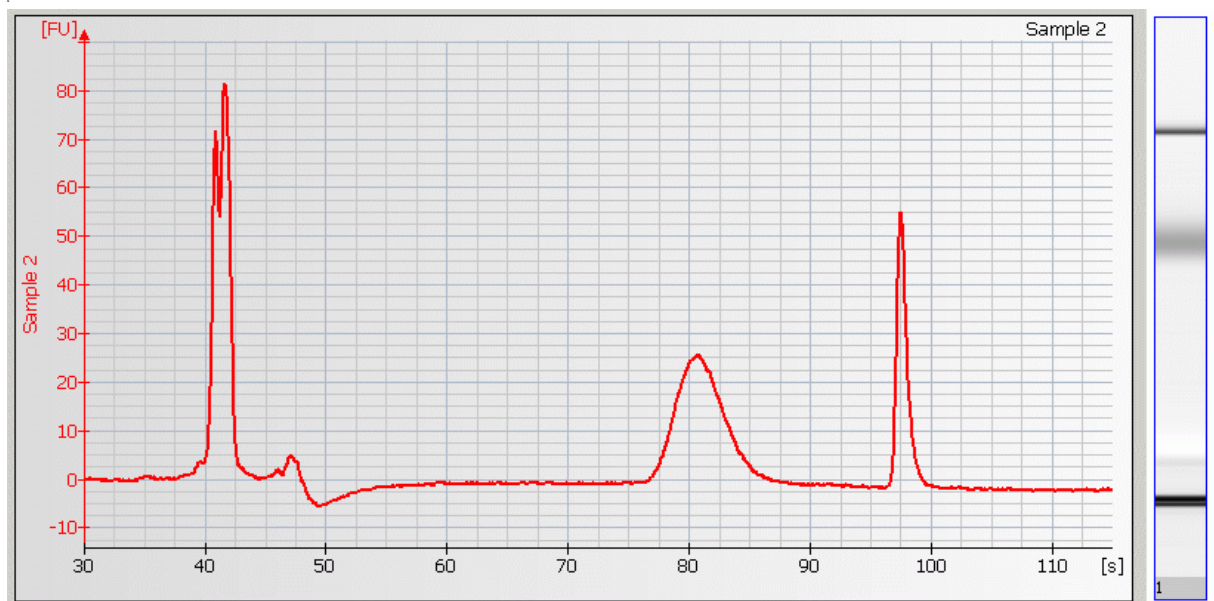
## Causes for Missing Upper Marker

Most Probable Causes	Solution
Alignment of upper marker not worked properly	Manually set the upper marker: <ol style="list-style-type: none"><li>1 In the electropherogram tab, highlight the appropriate peak in the peak table.</li><li>2 With a right-mouse click, select “Manually Set Upper Marker”.</li></ol>
Upper marker digested by restriction enzymes.	Inactivate restriction enzyme by adding EDTA or heat inactivate according to the manufacturers instruction.
Probable Causes	Solution
Too high salt concentration in sample	Refer to the maximum sample buffer salt concentration as specified in the reagent kit guide. Dilute samples with deionized DNase free water if necessary.

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## Broad Peaks

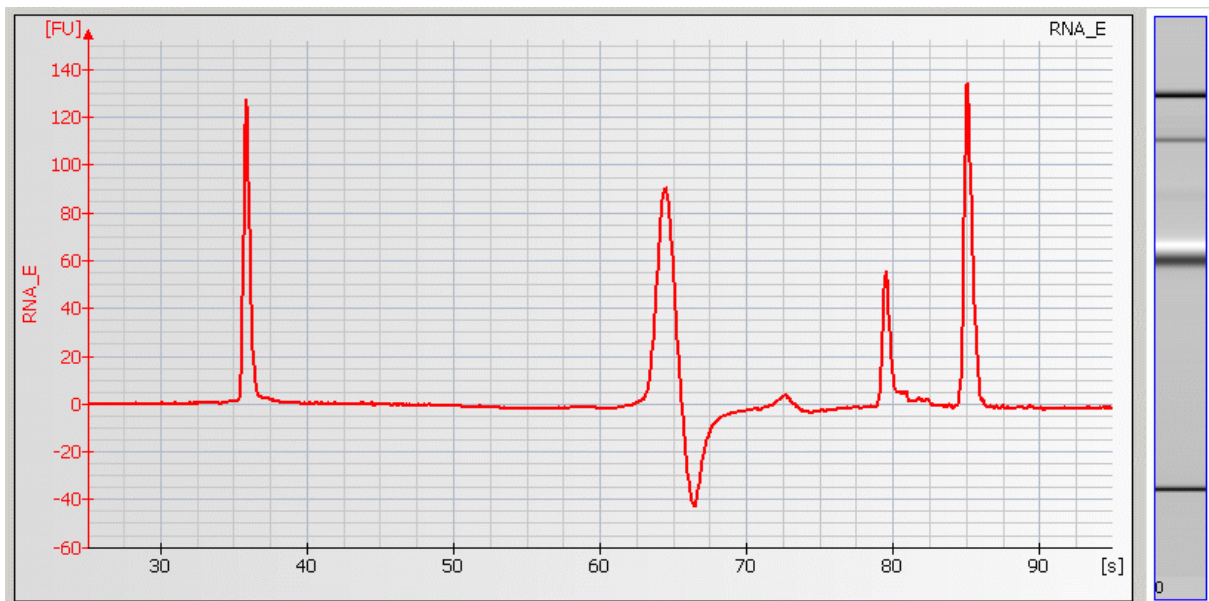


## Causes for Broad Peaks

Most Probable Causes	Solution
Current leaks due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.
Clogged gasket and plastic adapter of priming station	Check the priming station as described in <a href="#">“Checking the Chip Priming Station for Proper Performance - Seal Test”</a> on page 209. Clean/replace gasket and plastic adapter if necessary.
Wrong settings of the chip priming station.	Check if clip and base plate of priming station are in the right position (see Reagent Kit Guide).
Dye concentration too high.	Use dye concentration according to the Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix.
Probable Causes	Solution
Genomic DNA or cDNA contamination.	Check DNA preparation procedure.

Back to [“Symptoms \(DNA\)”](#) on page 41

# Baseline Dips

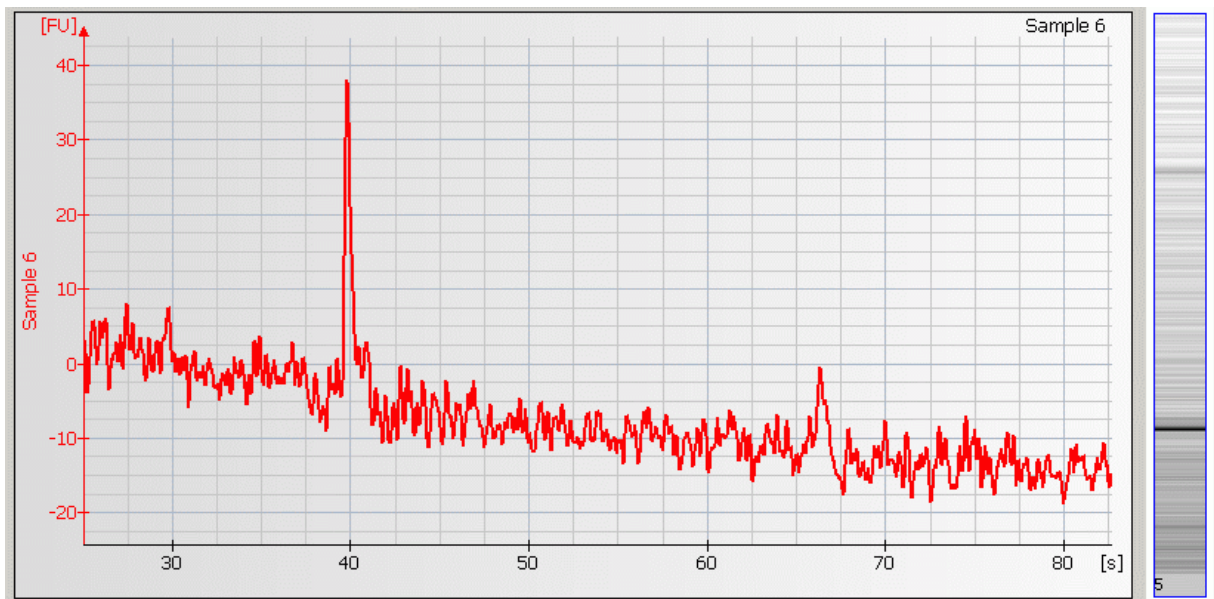


## Causes for Baseline Dips

Probable Causes	Solution
Too high sample concentration.	Use sample concentration according to the DNA Reagent Kit Guide.
Sample impurities: e.g. genomic DNA, ss DNA, etc.	Check DNA-isolation protocol. If possible, clean up samples.
Probable Causes	Solution
Dye concentration too low.	Use dye concentration according to the Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.
Least Probable Causes	Solution
Autofocus failure.	Check autofocus by means of the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If autofocus fails, contact Agilent Technologies.

Back to “[Symptoms \(DNA\)](#)” on page 41

# Baseline Noise

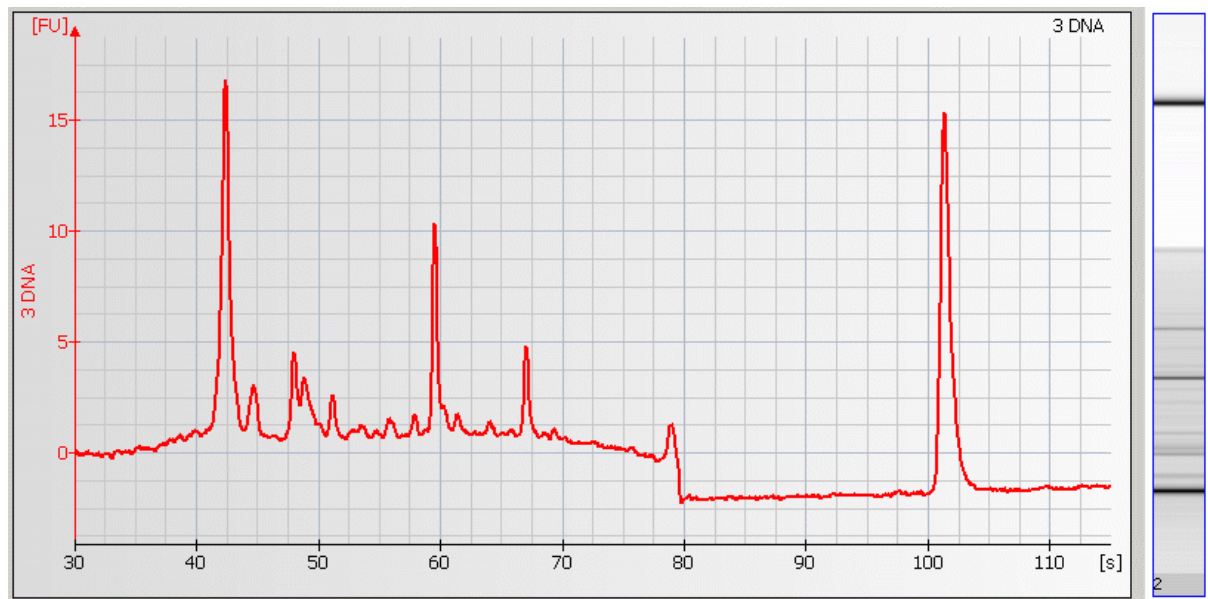


## Causes for Baseline Noise

Most Probable Causes	Solution
Fingerprint on focusing lens or on the backside of the chip.	Clean lens as described in “ <a href="#">Cleaning the Lens</a> ” on page 222.  Do not touch the underside of the chip.
Probable Causes	Solution
Autofocus failure or High voltage power supply defective	Check autofocus and high voltage power supply by means of the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If tests fail, contact Agilent Technologies.
Least Probable Causes	Solution
Chip contaminated.	Wear powder-free gloves only.  Don't touch the underside of the chip.  Don't touch the wells of the chip.  Clean the electrodes.  Load the chip immediately after taking it out of its sealed bag.

Back to “[Symptoms \(DNA\)](#)” on page 41

# Baseline Jumps



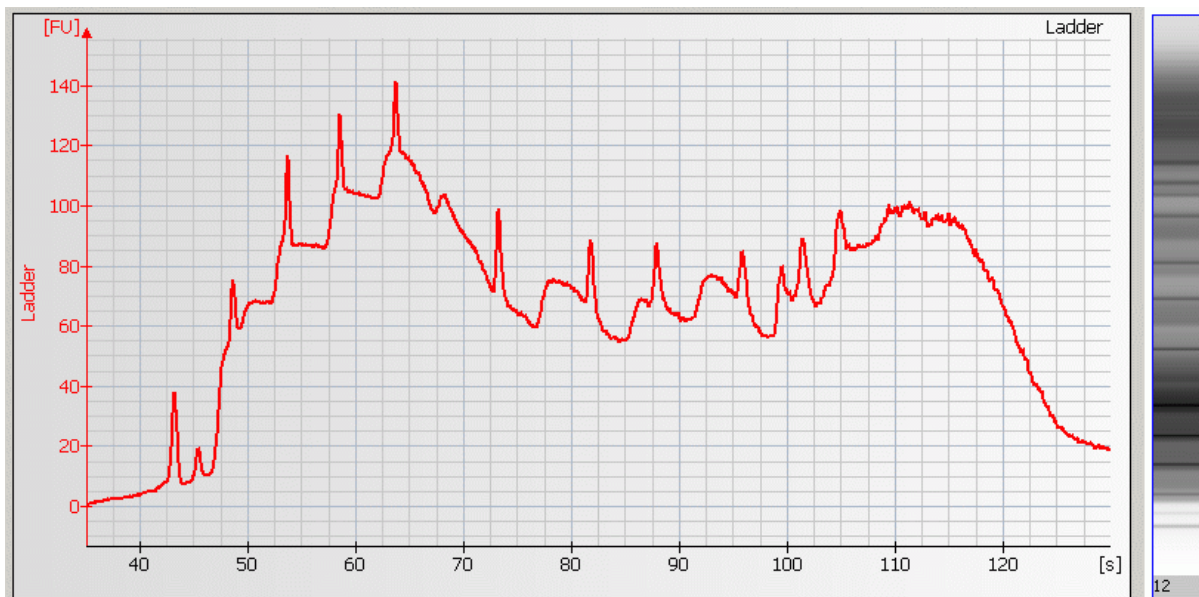
## Causes for Baseline Jumps

<b>Most Probable Causes</b>	<b>Solution</b>
Vibration of Agilent 2100 bioanalyzer.	Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Instrument lid was touched during the run.	Don't touch Agilent 2100 bioanalyzer during a run.
<b>Least Probable Causes</b>	<b>Solution</b>
Laser defective.	Check Laser by using the “ <a href="#">Hardware Diagnostics</a> ” on page 33.If the laser test fails, contact Agilent Technologies.

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# Wavy Baseline



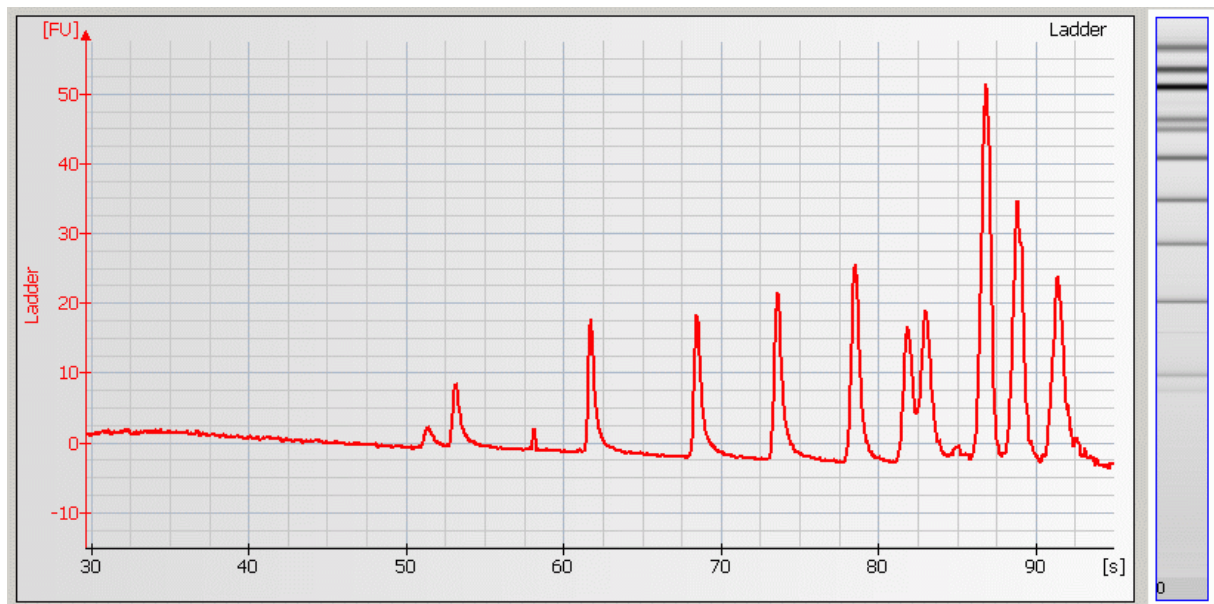
## Causes for Wavy Baseline

Most Probable Causes	Solution
Leak current due to dirty electrodes.	Clean electrodes as described in <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.
Detergents in PCR-buffer lowers surface tension in wells of chip. This leads to liquid spill on top of the chip during vortexing. As a result leak currents during the run will occur.	Prepare a new chip. Lower vortexing speed or mix samples manually.
Dye concentration too low.	Use dye concentration according to the Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix.

Probable Causes	Solution
Wrong settings of the chip priming station.	Check if clip and base plate of priming station are in the right position (see Reagent Kit Guide).
Clogged gasket and plastic adapter of priming station	Check the priming station as described in “ <a href="#">Checking the Chip Priming Station for Proper Performance - Seal Test</a> ” on page 209. Clean/replace gasket and plastic adapter if necessary.
Least Probable Causes	Solution
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 bioanalyzer in thermally stable environment.
High voltage power supply defective.	Check high voltage power supply using the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If the power supply is defective, contact Agilent Technologies.

Back to “[Symptoms \(DNA\)](#)” on page 41

# Late Migration



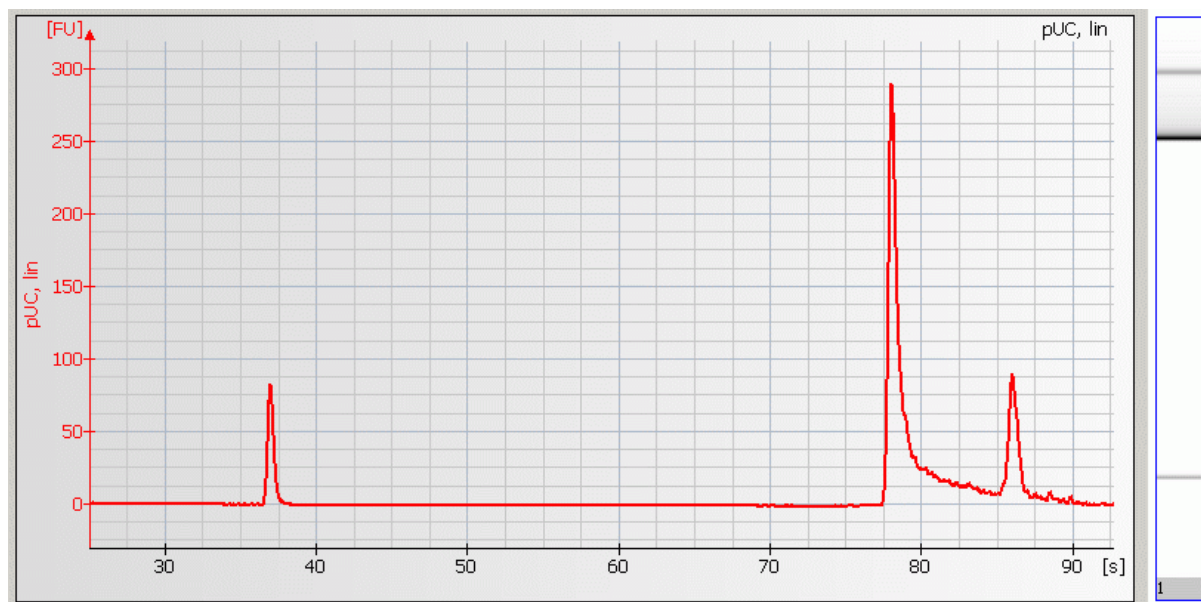
## Causes for Late Migration

Most Probable Causes	Solution
Vortex speed too high.	Vortex at lower (medium) speed. For chips use only the IKA vortexer.
Leak current due to dirty electrodes.	Clean electrodes as described in <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.
Detergents in PCR-buffer lowers surface tension in wells of chip. This leads to liquid spill on top of the chip during vortexing. As a result leak currents during the run will occur.	Prepare a new chip. Lower vortexing speed or mix samples manually.
Dye concentration too low.	Use dye concentration according to the Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix.
Probable Causes	Solution
Vortex adapter not connected tightly.	Press vortex adapter tightly on mount (vortex adapter must not rock).  Replace vortex adapter (p/n 5022-2190) as described in <a href="#">“Maintenance of the Vortexer: Changing the Adapter”</a> on page 228.

Clogged gasket and plastic adapter of priming station	Check chip priming station as described in “ <a href="#">Checking the Chip Priming Station for Proper Performance - Seal Test</a> ” on page 209). Clean/replace gasket and plastic adapter if necessary.
Wrong settings of the chip priming station.	Check if clip and base plate of priming station are in the right position (see Reagent Kit Guide).
Genomic DNA or high molecular weight DNA contamination.	Check DNA isolation protocol.
<b>Least Probable Causes</b>	<b>Solution</b>
Changes of ambient temperature of more than 5 °C during the run.	Place Agilent 2100 bioanalyzer in thermally stable environment.

Back to “[Symptoms \(DNA\)](#)” on page 41

# Peak Tailing



## Causes for Peak Tailing

### Most Probable Causes

Restriction enzymes may cause peak tailing.



### Solution

Use different restriction enzyme. Apply additional purification step.

Back to [“Symptoms \(DNA\)”](#) on page 41

# Troubleshooting the RNA Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the run log for the data file. Select the Log Book tab in the Data and Assay context. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 Bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see “[Symptoms \(RNA\)](#)” on page 82.

For most observations you will find at least one corresponding example, depicting a typical electropherogram or result table. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by priority.

The causes are grouped into three levels:

- most probable cause
- probable cause



- least probable cause

A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by probability.

## Symptoms (RNA)

Click the icon to see an example, or go straight to the troubleshooting hints.

- [“Residual Gel in Spin Filter after Centrifugation”](#) on page 83
- [“Too High Quantitation Results”](#) on page 84
- [“Too Low Quantitation Results”](#) on page 86
- [“Chip Not Detected”](#) on page 88
- [“Poor Chip Performance Error Message”](#) on page 89
-  [“Additional Sample or Ladder Peak”](#) on page 90
-  [“Degraded RNA Ladder and/or Samples”](#) on page 93
-  [“Spikes”](#) on page 95
-  [“Low Sensitivity”](#) on page 98
-  [“Baseline Noise”](#) on page 101
-  [“Broad Peaks”](#) on page 103
-  [“Missing Peaks”](#) on page 106
-  [“Causes for Missing RNA Fragment”](#) on page 110
-  [“Wavy Baseline”](#) on page 111
-  [“Cross Contamination”](#) on page 114
-  [“Late Migration of RNA Ladder or Samples”](#) on page 116

## Residual Gel in Spin Filter after Centrifugation

<b>Most Probable Causes</b>	<b>Solution</b>
Gel was centrifuged at too low g-value.	Refer to the RNA Reagent Kit Guides for proper centrifuge settings.
Cooled centrifuge was used for preparation of gel-dye mix and/or destaining solution.	Repeat centrifugation step without cooling.
<b>Least Probable Causes</b>	<b>Solution</b>
Particles in the gel-dye mix and/or destaining solution.	Repeat the preparation of the gel-dye mix and/or destaining solution. Wear powder-free gloves only.

Back to [“Symptoms \(RNA\)”](#) on page 82

## Too High Quantitation Results

Most Probable Causes	Solution
Pipetting error during preparation of ladder/samples.	Check dilution procedure and calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust speed to set-point.

Probable Causes	Solution
RNA ladder degraded.	Use new ladder aliquot/chip. Always wear gloves when handling chips/RNA samples to prevent them from getting contaminated. Follow decontamination procedure, for details refer to <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.
Electrodes contaminated with RNases.	Clean electrodes with RNaseZAP. Follow decontamination procedure, for details refer to <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192
Dye concentration too low.	Use dye concentration according to the RNA Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.
RNA ladder not denatured.	Heat denature the RNA ladder as described in the RNA Reagent Kit Guide.

Back to [“Symptoms \(RNA\)”](#) on page 82

## Too Low Quantitation Results

<b>Most Probable Causes</b>	<b>Solution</b>
Inaccurate reference measurement (e.g. UV-absorption) due to remaining UV absorbing contaminant in the sample.	Purify sample for UV measurement.
Pipetting error during preparation of mixtures.	Check dilution procedure and calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the speed to the set-point.
<b>Probable Causes</b>	<b>Solution</b>
RNA ladder not denatured.	Heat denature the RNA ladder as described in the RNA Reagent Kit Guide.
Sample concentration too high.	Use sample concentration according to the RNA Reagent Kit Guide.

Least Probable Causes	Solution
Dye concentration too high.	Use dye concentration according to the RNA Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.

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## Chip Not Detected

Most Probable Causes	Solution
Amount of liquid pipetted is too low or chip is empty.	Check assay procedure on amount of liquid to be pipetted. Pipette sample or buffer in all wells.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station as described in <a href="#">“Checking the Chip Priming Station for Proper Performance - Seal Test”</a> on page 209. Check if clip and base plate of priming station are in the correct position (see RNA Reagent Kit Guide).
Probable Causes	Solution
No communication between Agilent 2100 bioanalyzer and PC.	Test the PC-instrument communication as described in <a href="#">“Troubleshooting the Instrument Communication”</a> on page 15.
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the <a href="#">“Hardware Diagnostics”</a> on page 33. If the power supply is defective, contact Agilent Technologies.

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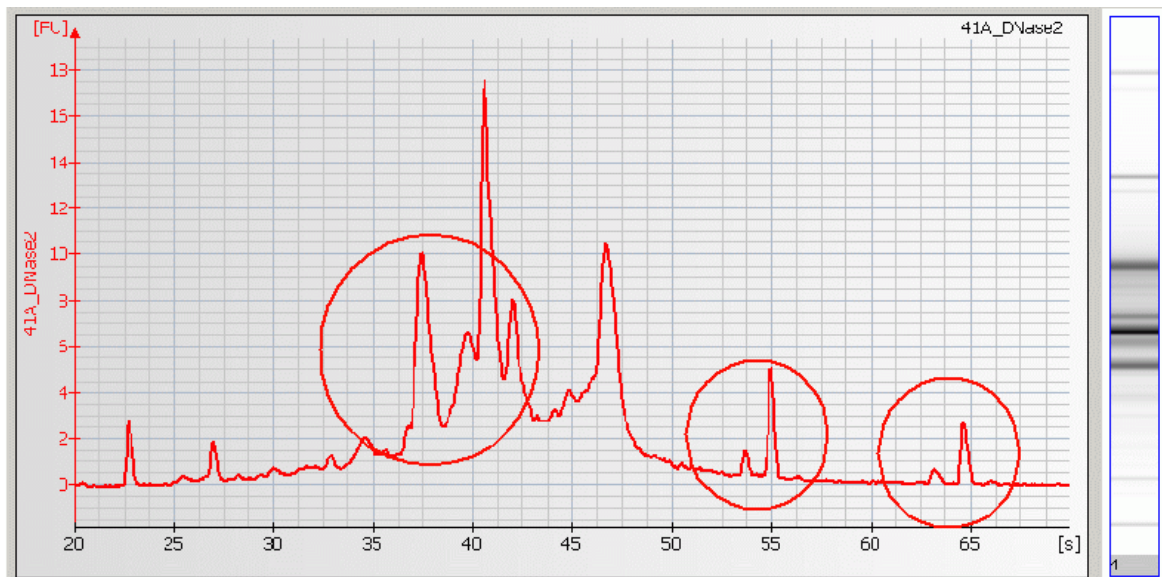


## Poor Chip Performance Error Message

Most Probable Causes	Solution
Amount of liquid pipetted is too low or one or more wells of the chip are empty.	Check Reagent Kit Guide on amount of liquid to be pipetted. Check calibration of pipette.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station as described in <a href="#">“Checking the Chip Priming Station for Proper Performance - Seal Test”</a> on page 209). Check if clip and base plate of priming station are in the correct position (see RNA Reagent Kit Guide).
Probable Causes	Solution
Chip preparation with cold reagents/chips.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes before use. Store chips at room temperature.
Least Probable Causes	Solution
High voltage power supply defective.	Check high voltage power supply using the <a href="#">“Hardware Diagnostics”</a> on page 33. If the power supply is defective, contact Agilent Technologies.

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## Additional Sample or Ladder Peak



## Causes for Additional Sample or Ladder Peaks

<b>Most Probable Causes</b>	<b>Solution</b>
Gel-dye mix expired.	Use prepared gel-dye mix within one day.
RNA ladder/sample not denaturated properly.	Heat ladder/samples at 70 °C for 2 minutes.
Particles in tubes.	For reagent preparation, use tubes that are supplied with the kit. Do not use autoclaved tubes.
Chip contaminated.	Wear powder-free gloves only.
Dust particles in separation channels.	Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
<b>Probable Causes</b>	<b>Solution</b>
Dye agglomerates present in the gel-dye mix.	Use dye concentration according to the Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes and vortex for 10 seconds before preparing the gel-dye mix. Protect the dye from light during this time. After centrifugation, the freshly prepared gel-dye should be taken up from the top of the tube.

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Chip preparation with cold reagents/chips.

Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes before use. Store chips at room temperature.

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<b>Least Probable Causes</b>	<b>Solution</b>
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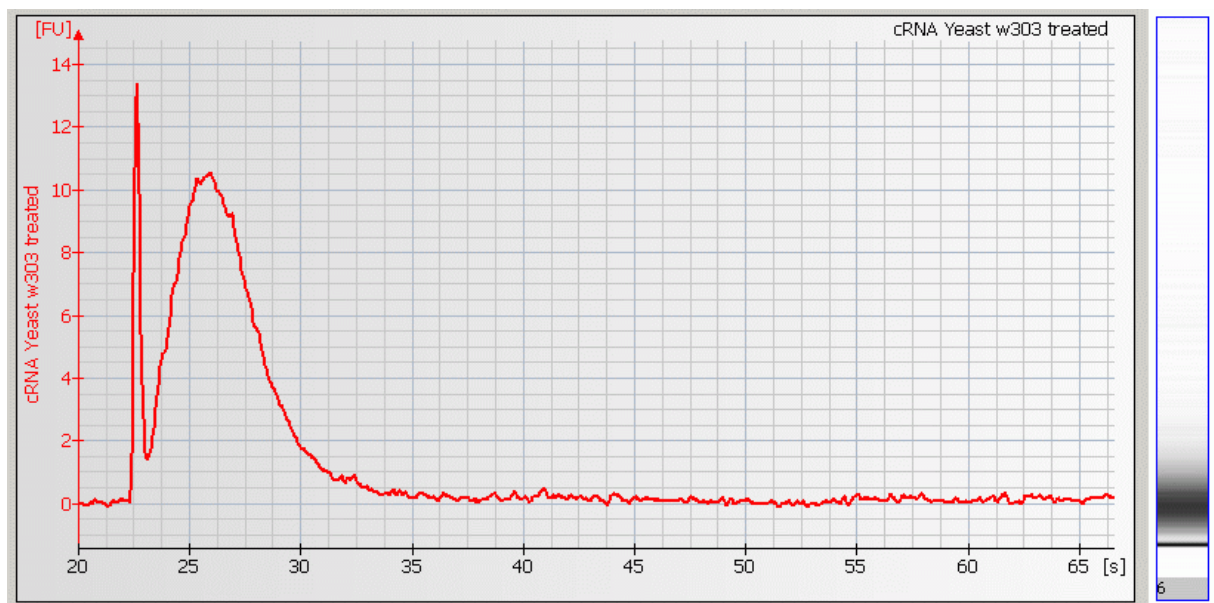
RNA ladder/sample degraded.	
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Always wear gloves when handling chips/RNA samples to prevent them from getting contaminated. Follow decontamination procedure, see <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.
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## Degraded RNA Ladder and/or Samples

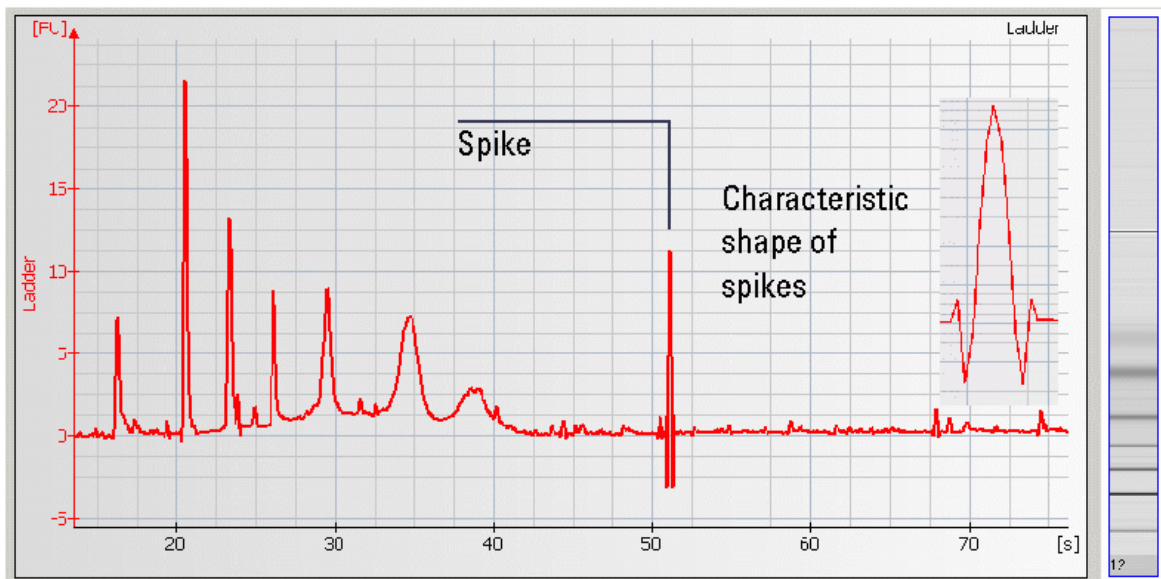


## Causes for Degraded RNA Ladder and/or Samples

Most Probable Causes	Solution
RNase contamination of the pin-set.	Decontaminate the pin-set. Follow decontamination procedure, see “ <a href="#">How to clean the Pin Set of the Electrode Cartridge</a> ” on page 192.
RNase contamination of chips and/or reagents.	Use a new chip and/or fresh reagents. Wear powder-free gloves when preparing the chip.

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# Spikes



## Causes for Spikes

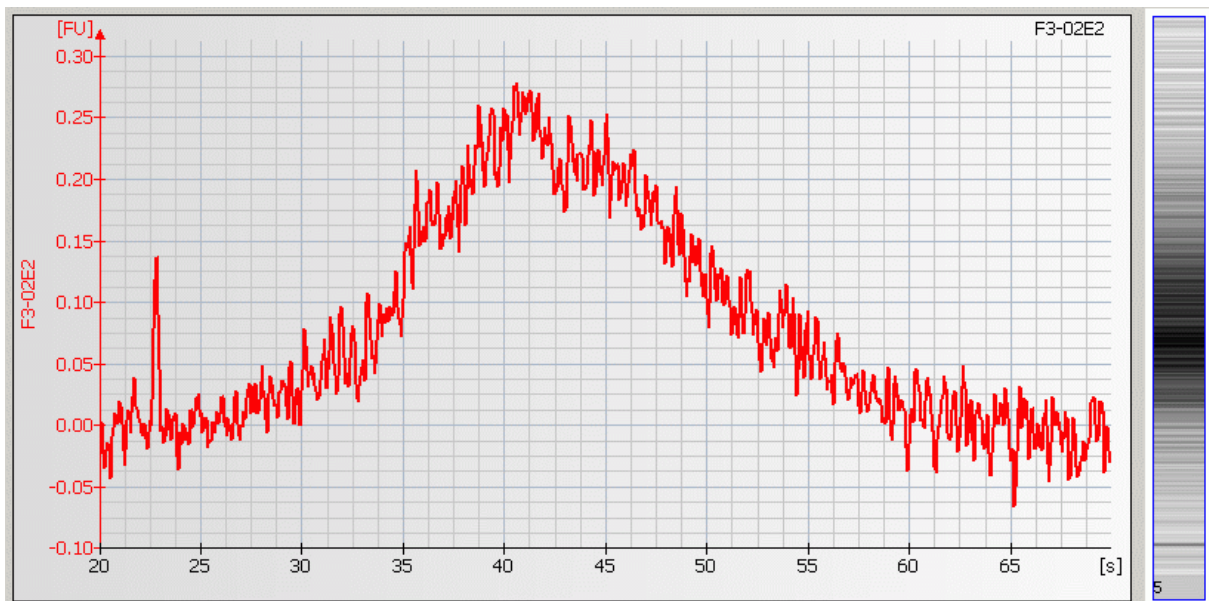
Most Probable Causes	Solution
Vibration of Agilent 2100 bioanalyzer.	Don't touch Agilent 2100 bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Particles in tubes.	For reagent preparation, use tubes that are supplied with the kit. Do not use autoclaved tubes.
Chip/gel-dye mix contaminated.	Prepare new chip with new gel-dye mix: Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.



<b>Probable Causes</b>	<b>Solution</b>
Dye agglomerates present in the gel-dye mix.	Use dye concentration according to the Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes and vortex for 10 seconds before preparing the gel-dye mix. Protect the dye from light during this time. After centrifugation, the freshly prepared gel-dye should be taken up from the top of the tube.
<b>Least Probable Causes</b>	<b>Solution</b>
Power outlett	Install power filter.

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# Low Sensitivity



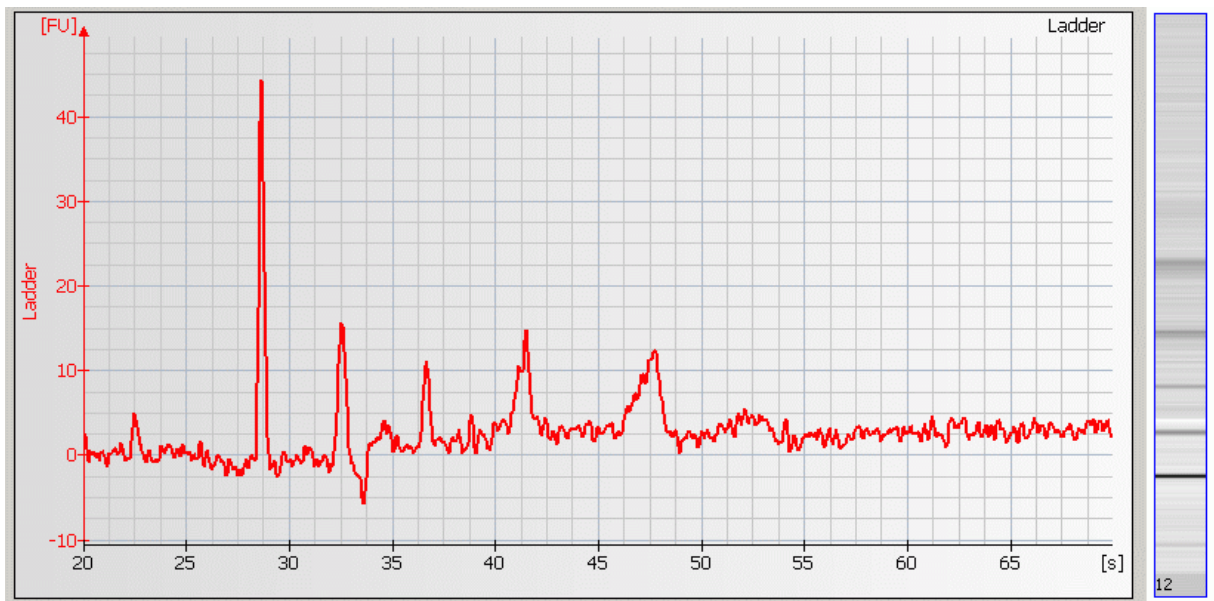
## Causes for Low Sensitivity

<b>Most Probable Causes</b>	<b>Solution</b>
Gel-dye mix expired.	Use prepared gel-dye mix within one day.
Dye concentration too low.	Use dye concentration according to the Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.
Pipetting error during preparation of mixtures.	Check dilution procedure and calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
<b>Probable Causes</b>	<b>Solution</b>
Fingerprint on focusing lens or on the backside of the chip.	Clean lens as described in <a href="#">“Cleaning the Lens”</a> on page 222.  Do not touch the underside of the chip
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use IKA shaker for chip vortexing. Adjust speed to set-point (2400 rpm).

Least Probable Causes	Solution
Autofocus or laser failure.	Check autofocus and laser using the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If tests fail, contact Agilent Technologies.

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# Baseline Noise

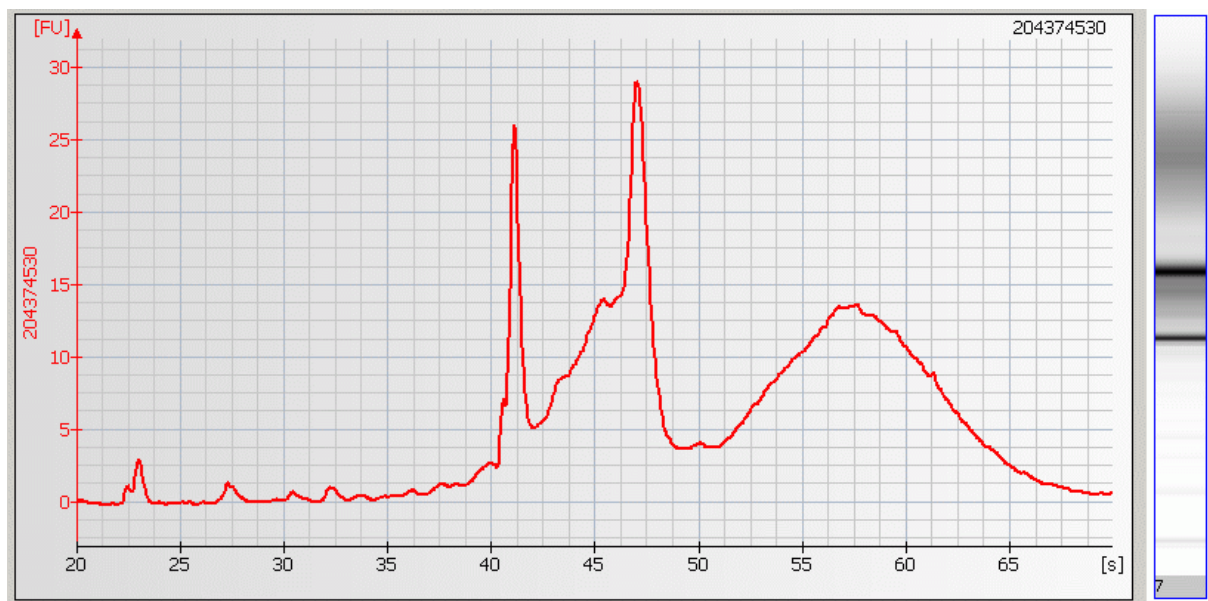


## Causes for Baseline Noise

Most Probable Causes	Solution
Fingerprint on focusing lens or on the backside of the chip.	Clean lens as described in <a href="#">“Cleaning the Lens”</a> on page 222.  Do not touch the underside of the chip
Chip contaminated.	Wear powder-free gloves only.  Don't touch the underside of the chip.  Don't touch the wells of the chip.  Clean the electrodes.  Load the chip immediately after taking it out of its sealed bag.
Vibration of Agilent 2100 bioanalyzer.	Don't touch Agilent 2100 bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Probable Causes	Solution
Dye concentration too low.	Use dye concentration according to the RNA Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.

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## Broad Peaks



## Causes for Broad Peaks

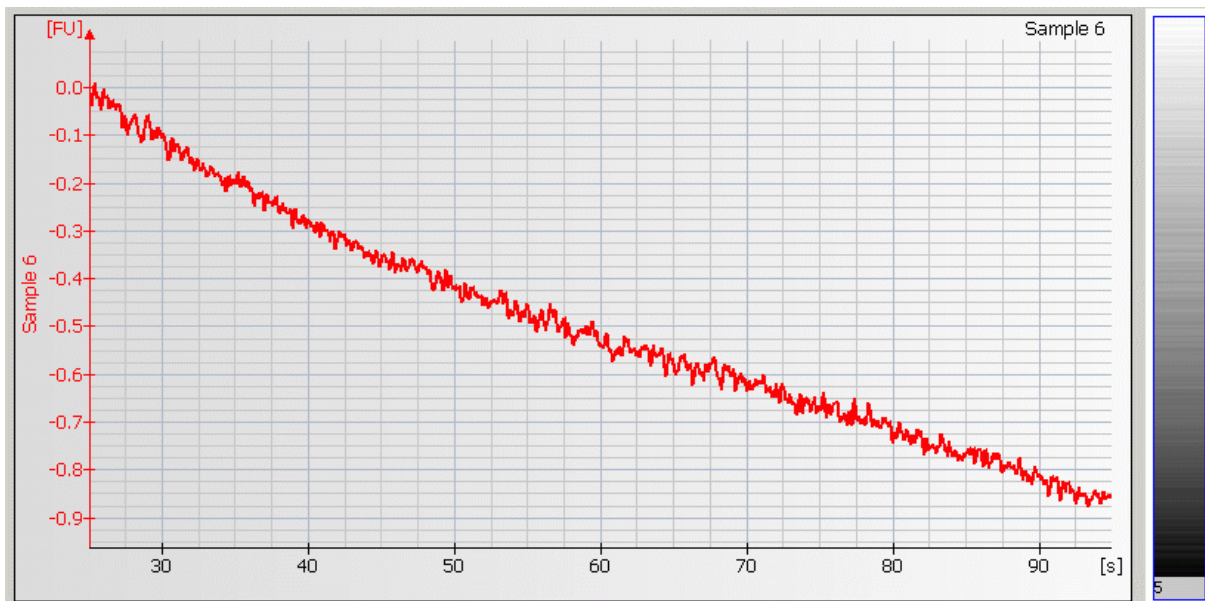
<b>Most Probable Causes</b>	<b>Solution</b>
Sample contaminated with genomic DNA.	Check RNA-isolation protocol. To remove genomic DNA, perform DNase treatment.
Leaks currents due to contaminated pin-set.	Clean the pin-set of the electrode cartridge. Follow cleaning procedure, see <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192
Electrodes contaminated with RNases.	Clean electrodes with RNaseZAP. Follow cleaning procedure, see <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192
<b>Probable Causes</b>	<b>Solution</b>
Clogged gasket and plastic adapter of priming station	Check the priming station as described in <a href="#">“Checking the Chip Priming Station for Proper Performance - Seal Test”</a> on page 209. Clean/replace gasket and plastic adapter if necessary.
Wrong settings of chip priming station.	Check if clip and base plate of priming station are in the right position (see RNA Reagent Kit Guide).



Least Probable Causes	Solution
Dye concentration too high.	Use dye concentration according to the Reagent Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.

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# Missing Peaks



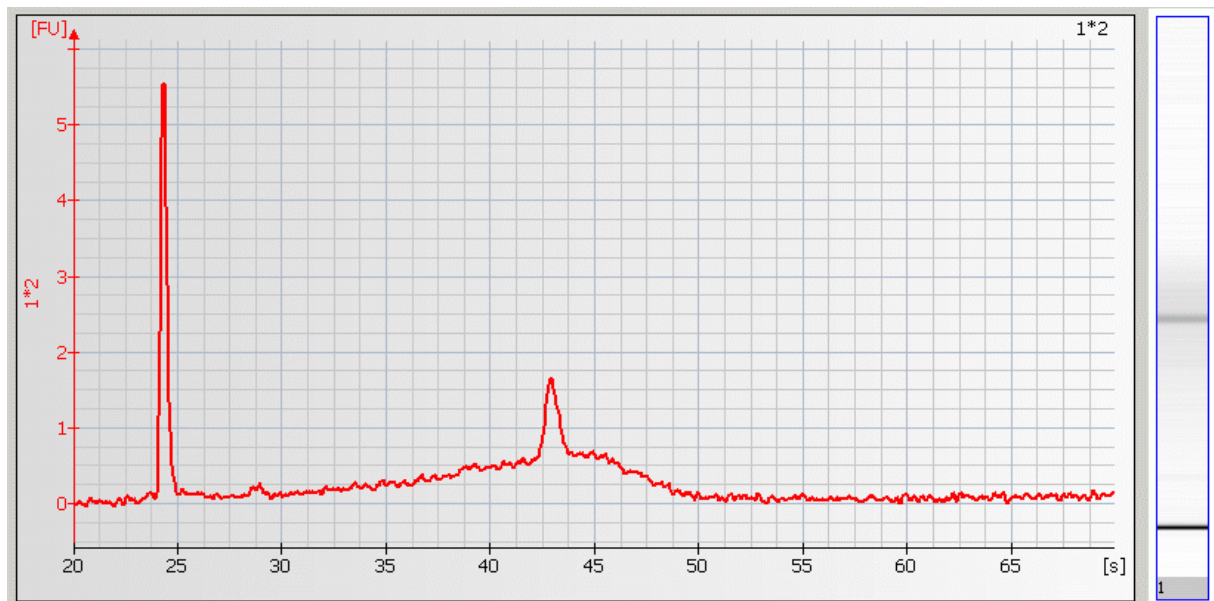
## Causes for Missing Peaks

Most Probable Causes	Solution
Too high salt concentration in sample	Refer to the maximum sample buffer salt concentration as specified in the reagent kit guide. Dilute samples with deionized RNase free water if necessary.
Clogged gasket and plastic adapter of priming station	Check chip priming station as described in <a href="#">“Checking the Chip Priming Station for Proper Performance - Seal Test”</a> on page 209). Clean/replace gasket and plastic adapter if necessary.
Wrong settings of the chip priming station.	Check if clip and base plate of priming station are in the right position (see Reagent Kit Guide).
Leak current due to contaminated electrodes or wet chip surface (detergents in RNA elution buffer)	Clean electrodes with analysis-grade water and a toothbrush, see <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.  Prepare a new chip. Lower vortexing speed or mix samples manually

<b>Probable Causes</b>	<b>Solution</b>
Laser broken.	Perform Laser/LED/Optics test and Autofocus test as described in “ <a href="#">Hardware Diagnostics</a> ” on page 33. If tests fail contact Agilent Technologies.
<b>Least Probable Causes</b>	<b>Solution</b>
Autofocus failure or high voltage power supply defective	Check autofocus and high voltage power supply by means of the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If diagnostic fails, contact Agilent Technologies.
Sample wells filled with water.	Refer to the Reagent Kit Guide for proper preparation of the chip.

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# Missing RNA Fragment

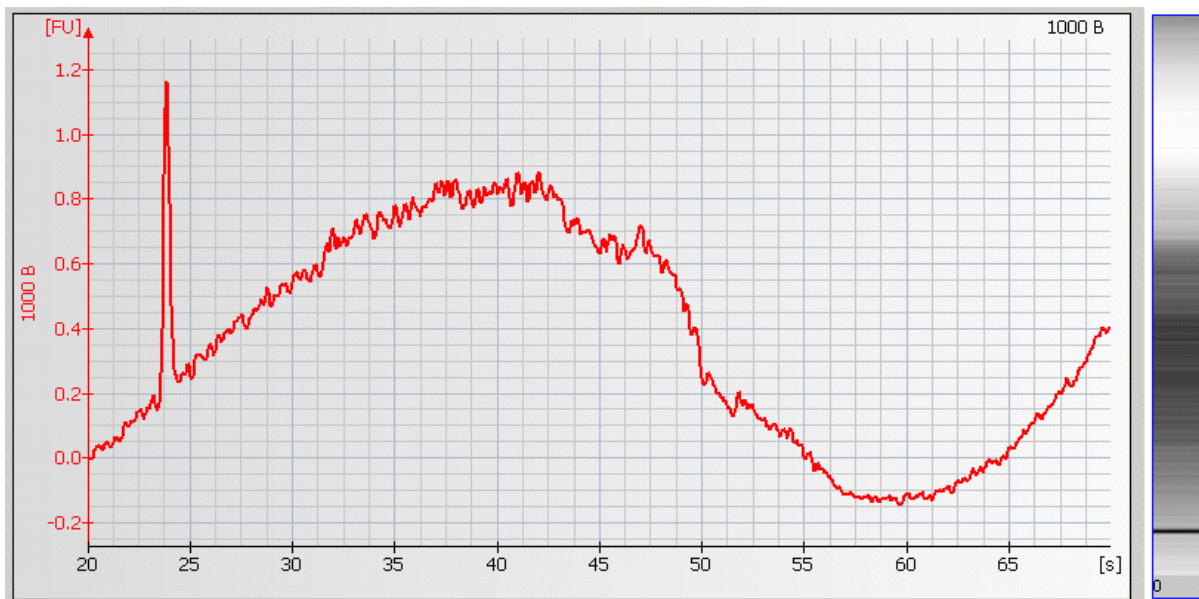


## Causes for Missing RNA Fragment

Most Probable Causes	Solution
Too high salt concentration in sample.	Refer to the maximum sample buffer salt concentration as specified in the reagent kit guide, Dilute samples with deionized RNase free water if necessary,
Probable Causes	Solution
Sample degradation because of RNase-contamination of electrodes or reagents.	Clean electrodes with RNaseZAP. Follow cleaning procedure, see <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.  Use a new chip and/or fresh reagents. Wear powder-free gloves when preparing the chip.
Incompatible column material of RNA extraction kit.	Use RNA extraction kit of different vendor.

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# Wavy Baseline



## Causes for Wavy Baseline

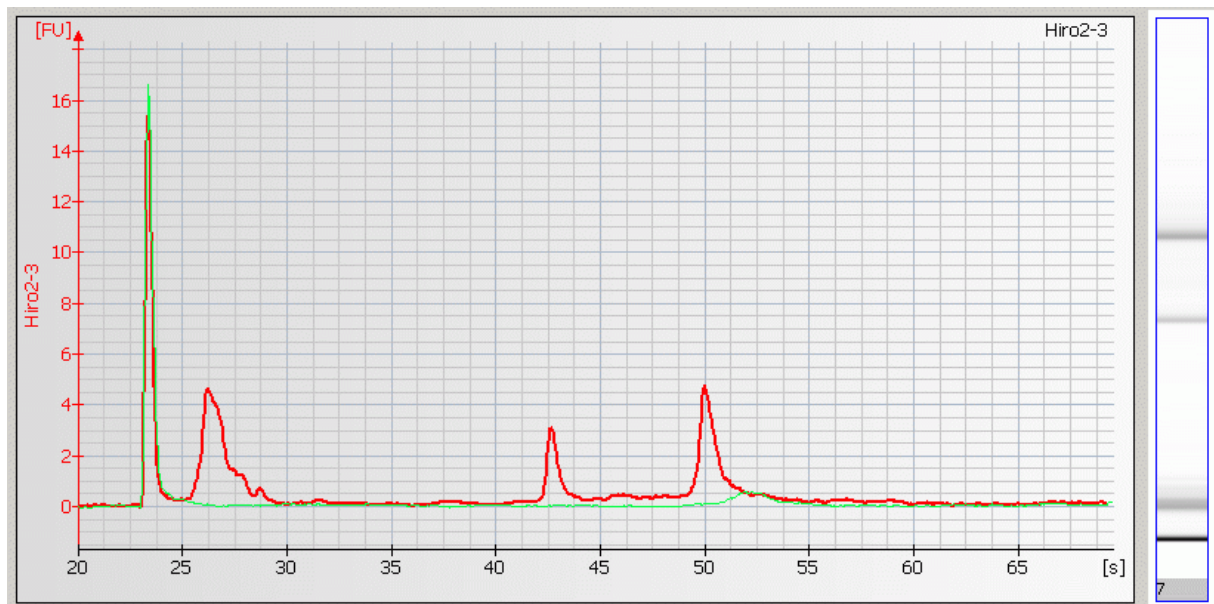
Most Probable Causes	Solution
Following sample contaminated with genomic DNA causes baseline fluctuations.	Check RNA-isolation protocol. To remove genomic DNA, perform DNase treatment.
Leak current due to contaminated electrodes or wet chip surface (detergents in RNA elution buffer).	Clean electrodes with analysis-grade water and a toothbrush, see <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.  Prepare a new chip. Lower vortexing speed or mix samples manually
Clogged gasket and plastic adapter of priming station	Check chip priming station as described in <a href="#">“Checking the Chip Priming Station for Proper Performance - Seal Test”</a> on page 209). Clean/replace gasket and plastic adapter if necessary.
Contaminated electrode cartridge.	Clean the electrode cartridge as described in <a href="#">“RNA Nano Assay”</a> on page 184 or <a href="#">“RNA Pico Assay”</a> on page 188.



<b>Probable Causes</b>	<b>Solution</b>
Wrong settings of the chip priming station.	Check if clip and base plate of priming station are in the right position (see Reagent Kit Guide).
<b>Least Probable Causes</b>	<b>Solution</b>
Autofocus failure or high voltage power supply defective	Check autofocus and high voltage power supply by means of the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If diagnostic fails, contact Agilent Technologies.

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# Cross Contamination

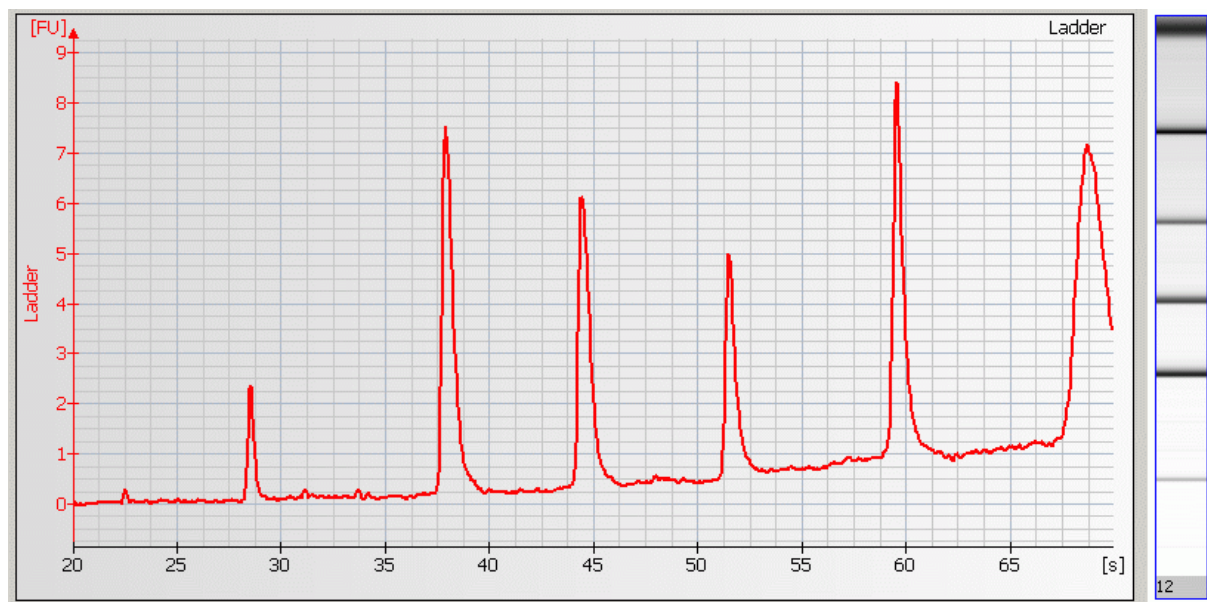


## Causes for Cross Contamination

Most Probable Causes	Solution
Contamination of pipette tips.	Use fresh tips for each pipetting step.
Contamination of electrodes.	Clean electrodes between runs like described in " <a href="#">Maintenance of the Electrode Cartridge</a> " on page 180.
Least Probable Causes	Solution
Leaks currents due to contaminated pin-set.	Clean the pin-set of the electrode cartridge. Follow cleaning procedure, see " <a href="#">How to clean the Pin Set of the Electrode Cartridge</a> " on page 192.

Back to "[Symptoms \(RNA\)](#)" on page 82

## Late Migration of RNA Ladder or Samples



## Causes for Late Migration of RNA Ladder or Sample



Most Probable Causes	Solution
Vortex speed too high.	Vortex at lower (medium) speed. For chips use only the IKA vortexer.
Leak current due to dirty electrodes.	Clean electrodes as described in <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.
Detergents in RNA elution buffer lowers surface tension in wells of chip. This leads to liquid spill on top of the chip during vortexing. As a result leak currents during the run will occur.	Prepare a new chip. Lower vortexing speed or mix samples manually.
Probable Causes	Solution
Vortex adapter not connected tightly.	Press vortex adapter tightly on mount (vortex adapter must not rock).  Replace vortex adapter (p/n 5022-2190) as described in <a href="#">“Maintenance of the Vortexer: Changing the Adapter”</a> on page 228.

Probable Causes	Solution
Clogged gasket and plastic adapter of priming station	Check chip priming station as described in “ <a href="#">Checking the Chip Priming Station for Proper Performance - Seal Test</a> ” on page 209). Clean/replace gasket and plastic adapter if necessary.

Back to “[Symptoms \(RNA\)](#)” on page 82

# Troubleshooting the Protein Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the Run Log for the data file. Select the Log Book tab in the Data and Assay context. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see “[Symptoms \(Protein\)](#)” on page 121.

For most observations you will find at least one corresponding example, depicting a typical electropherogram or result table. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by priority.

The causes are grouped into three levels:

- most probable cause
- probable cause

- least probable cause

A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by probability.



## Symptoms (Protein)

Click the icon to see an example, or go straight to the troubleshooting hints.

- [“Residual Gel in Spin Filter after Centrifugation”](#) on page 123
- [“Too High Quantitation Results”](#) on page 124
- [“Too Low Quantitation Results”](#) on page 126
- [“Wrong Sizing Result”](#) on page 128
- [“Chip Not Detected”](#) on page 130
- [“Poor Chip Performance Error Message”](#) on page 132
-  [“Short Run Time”](#) on page 134
-  [“Additional Sample or Ladder Peaks”](#) on page 136
-  [“Low or Missing Upper Marker in Ladder”](#) on page 139
-  [“Low or Missing Upper Marker in Sample”](#) on page 141
-  [“High Lower Marker Variability”](#) on page 144
-  [“Missing Peaks”](#) on page 146
-  [“Spikes”](#) on page 148
-  [“Poor Reproducibility”](#) on page 151
-  [“Low Sensitivity”](#) on page 154
-  [“Low Ladder Peaks”](#) on page 157
-  [“Broad Peaks”](#) on page 159

-  “Baseline Dips” on page 162
-  “Late Migration” on page 164

## Residual Gel in Spin Filter after Centrifugation

<b>Most Probable Causes</b>	<b>Solution</b>
Gel was centrifuged at too low g-value.	Refer to the protein reagent kit guide for proper centrifuge settings.
Cooled centrifuge was used for preparation of gel-dye mix and/or destaining solution.	Repeat centrifugation step without cooling.
<b>Least Probable Causes</b>	<b>Solution</b>
Particles in the gel-dye mix and/or destaining solution	Repeat the preparation of the gel-dye mix and/or destaining solution. Wear powder-free gloves only.

Back to [“Symptoms \(Protein\)”](#) on page 121

## Too High Quantitation Results

Most Probable Causes	Solution
Upper marker wrongly assigned.	Check assignment of upper marker. For details, please refer to the Online Help or Users Guide.
Diluted samples are degraded.	Use diluted samples within one day. Store samples at 4 °C when not in use for longer than 1 hour.
Sample buffer and/or Denaturing Solution not handled according to the instructions.	For proper preparation and storage of the sample buffer and denaturing solution, refer to the Protein Reagent Kit Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure and calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.

<b>Probable Causes</b>	<b>Solution</b>
Upper marker is degraded by proteases in the sample.	Treat the sample with protease inhibitors prior to the sample preparation.
High salt concentration in the sample	Check maximum salt concentration that are stated in the compatible buffer list in the reagent kit guide. Dilute the sample prior to the sample preparation or use a different buffer if possible.
Samples not completely denaturated.	Use fresh sample aliquot. Heat sample/ denaturing solution for 5 min at 100 °C.
Sample/denaturing solution are dried out.	Sample/denaturing solution were denaturated in 1.5 ml tubes. Use 0.5 ml tubes for denaturing.
<b>Least Probable Causes</b>	<b>Solution</b>
Loaded chip kept for too long before run.	Prepared chips must be used immediately.

Back to [“Symptoms \(Protein\)”](#) on page 121

## Too Low Quantitation Results

<b>Most Probable Causes</b>	<b>Solution</b>
Upper marker wrongly assigned.	Check assignment of upper marker. For details, please refer to the Online Help or Users Guide.
Pipetting error during preparation of mixtures.	Check dilution procedure and calibration of pipette.
Chip pipetting error.	Use new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
<b>Probable Causes</b>	<b>Solution</b>
Sample concentration too high.	Use sample concentration according to the specifications in the protein reagent kit guides. Don't forget to dilute samples with deionized water after heat denaturation.
Diluted sample are degraded.	Use diluted samples within one day. Store samples at 4 °C when not in use for longer than 1 hour.
<b>Lease Probable Causes</b>	<b>Solution</b>
Loaded chip kept too long before run.	Prepared chips must be used immediately.

Back to [“Symptoms \(Protein\)”](#) on page 121

## Wrong Sizing Result

<b>Most Probable Causes</b>	<b>Solution</b>
Ladder peaks wrongly assigned.	Check assignment of ladder peaks. For details, please refer to the Online Help or Users Guide.
Upper and/or lower marker wrongly assigned.	Store Sample Buffer/Denaturing Solution according to the instructions given in the reagent kit guide. Check assignment of markers. For details, please refer to the Online Help or Users Guide. See also " <a href="#">Low or Missing Upper Marker in Ladder</a> " on page 139.
Ladder degraded.	Use diluted ladder within one day. Store ladder at 4 °C when not in use for longer than 1 hour.
<b>Probable Causes</b>	<b>Solution</b>
Protein ladder not properly denaturated.	Use fresh ladder aliquot. Heat ladder for 5 min at 100 °C. Use 0.5 ml tubes for denaturating.



<b>Least Probable Causes</b>	<b>Solution</b>
Sample not reduced properly.	Some proteins will not migrate according to their molecular weight if they are not reduced properly due to internal di-sulfide bridges. Protein will migrate at higher molecular weights than expected. Check preparation of denaturing solution described in the reagent kit guide.
Protein characteristics	Glycosylation and other post-translational modifications may disturb micelle formation around the protein. Proteins will migrate a higher molecular weight than expected. However this effect is reproducible.

Back to [“Symptoms \(Protein\)”](#) on page 121

## Chip Not Detected

<b>Most Probable Causes</b>	<b>Solution</b>
Amount of liquid pipetted is too low or chip is empty.	Check assay procedure on amount of liquid to be pipetted. Pipette sample or buffer in all wells.
Air bubbles in the bottom of the well.	Always insert the pipette tip to the bottom of the well when dispensing the liquid. Remove large air bubbles with a pipette tip (small bubbles on top of well do not affect the assay).
<b>Probable Causes</b>	<b>Solution</b>
No communication between Agilent 2100 bioanalyzer and PC.	Test the PC-instrument communication as described in <a href="#">“Troubleshooting the Instrument Communication”</a> on page 15.
Chip not properly primed. Air bubble in chip.	Use a new chip. Check chip priming station as described in <a href="#">“Checking the Chip Priming Station for Proper Performance - Seal Test”</a> on page 209). Check if clip and base plate of priming station are in the correct position (see reagent kit guide).
<b>Least Probable Causes</b>	<b>Solution</b>
High voltage power supply defective.	Check high voltage power supply using the <a href="#">“Hardware Diagnostics”</a> on page 33. If the power supply is defective, contact Agilent Technologies.

Back to [“Symptoms \(Protein\)”](#) on page 121

## Poor Chip Performance Error Message

Most Probable Causes	Solution
Amount of liquid pipetted is too low or one or more wells of the chip are empty.	Check reagent kit guide on amount of liquid to be pipetted. Fill unused wells with ladder or sample replicate. Check calibration of pipette.
Air bubbles in the bottom of the well.	Always insert the pipette tip to the bottom of the well when dispensing the liquid. Remove large air bubbles with a pipette tip (small bubbles on top of well do not affect the assay).
Probable Causes	Solution
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station as described in “ <a href="#">Checking the Chip Priming Station for Proper Performance - Seal Test</a> ” on page 209. Check if clip and base plate of priming station are in the correct position (see protein reagent kit guide)

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Chip preparation was done with cold reagents/chip.

Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature before use. Store chips at room temperature.

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<b>Least Probable Causes</b>	<b>Solution</b>
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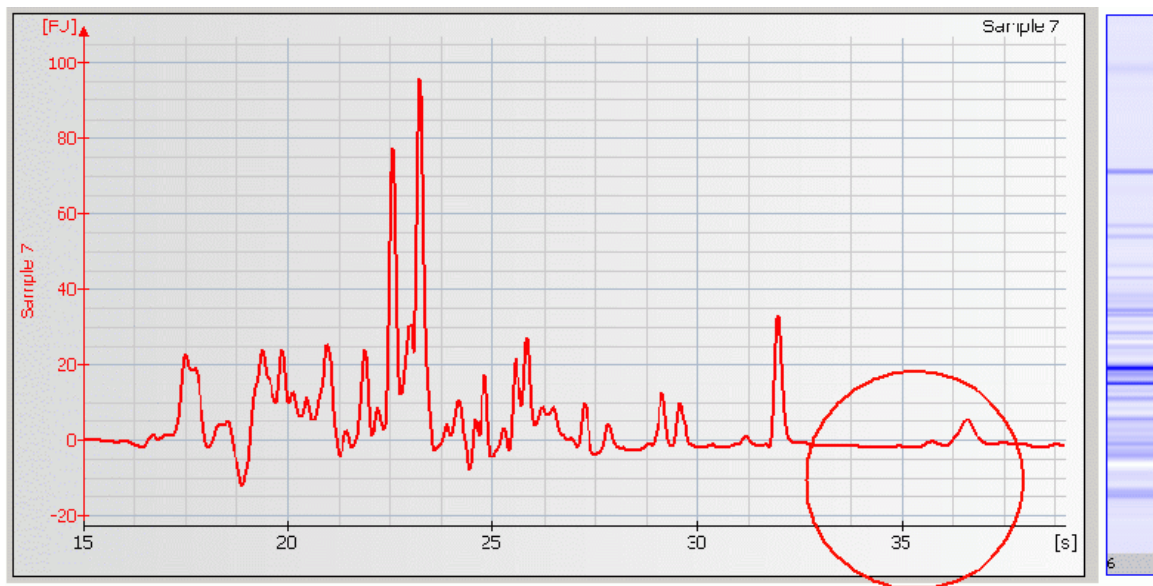
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High voltage power supply defective.	Check high voltage power supply using the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If the power supply is defective, contact Agilent Technologies.
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Back to “[Symptoms \(Protein\)](#)” on page 121

## Short Run Time

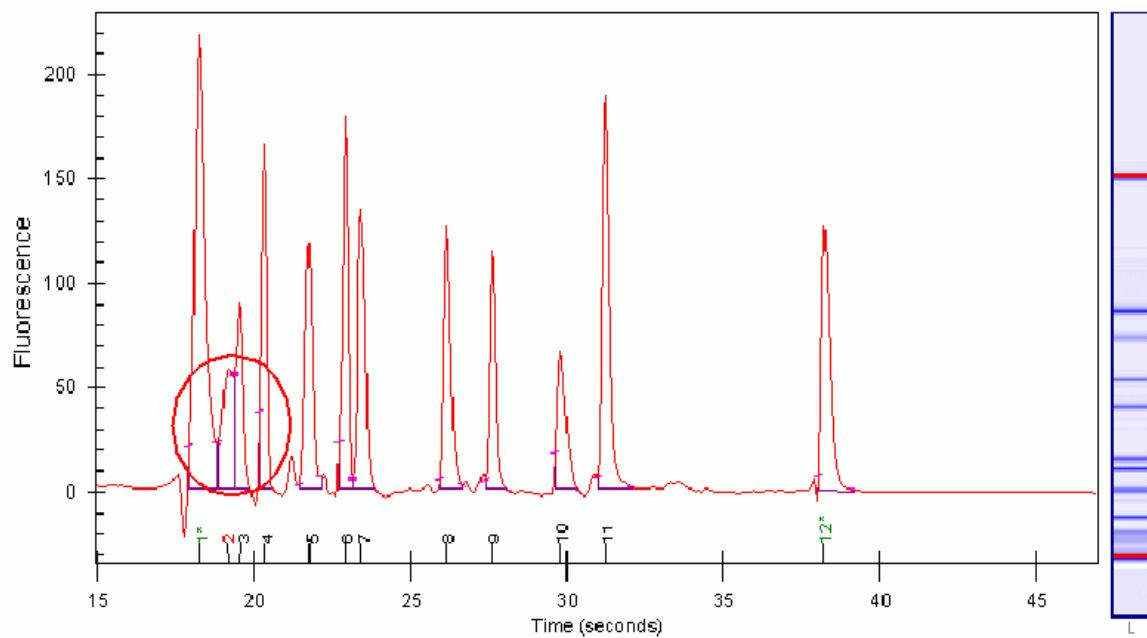


## Causes for Short Run Time

Most Probable Causes	Solution
<p>Upper marker in ladder was not correctly assigned due to low intensity of upper marker in the ladder.</p>	<p>To correct for wrong selected upper marker in ladder, set upper marker manually. If necessary, adjust peak find settings. If peaks are detected that are not part of the ladder, exclude them. For better upper marker identification:</p> <p>Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker. For details please refer to the Online Help or Users Guide.</p> <p>See also “<a href="#">Low or Missing Upper Marker in Ladder</a>” on page 139 for probable causes.</p>

Back to “[Symptoms \(Protein\)](#)” on page 121

## Additional Sample or Ladder Peaks



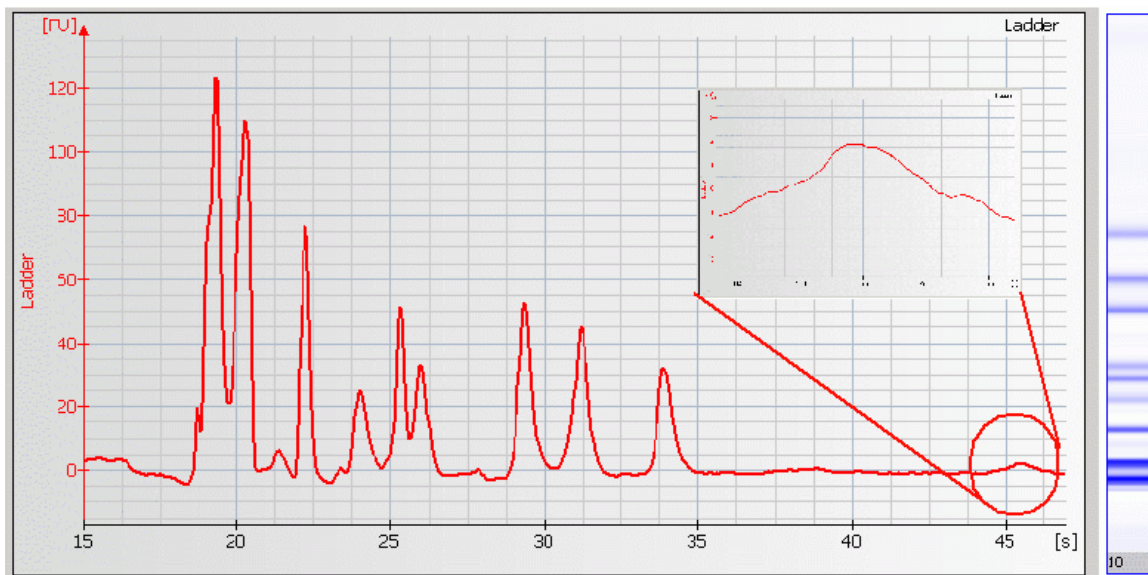


## Causes for Additional Sample or Ladder Peaks

Most Probable Causes	Solution
Sample or ladder not denaturated properly.	Use fresh sample aliquot. Heat sample/ denaturating solution and ladder for 5 min at 100 °C
Sample/denaturating solution and/or ladder are dried out during denaturation.	Sample/denaturating solution and/or ladder were denaturated in 1.5 ml tubes. Use 0.5 ml tubes for denaturating
Chip contaminated. Dust particles in separation channels.	Wear powder-free gloves only. Don't touch the wells of the chip.  Clean the electrodes: see <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192 for additional information.  Load the chip immediately after taking it out of its sealed bag.
Probable Causes	Solution
Ladder degraded.	Refer to the protein reagent kit guide for proper ladder storage. Optional: Prepare ladder aliquots and use a new aliquot.
Vibration of Agilent 2100 bioanalyzer.	Don't touch Agilent 2100 bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.

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## Low or Missing Upper Marker in Ladder

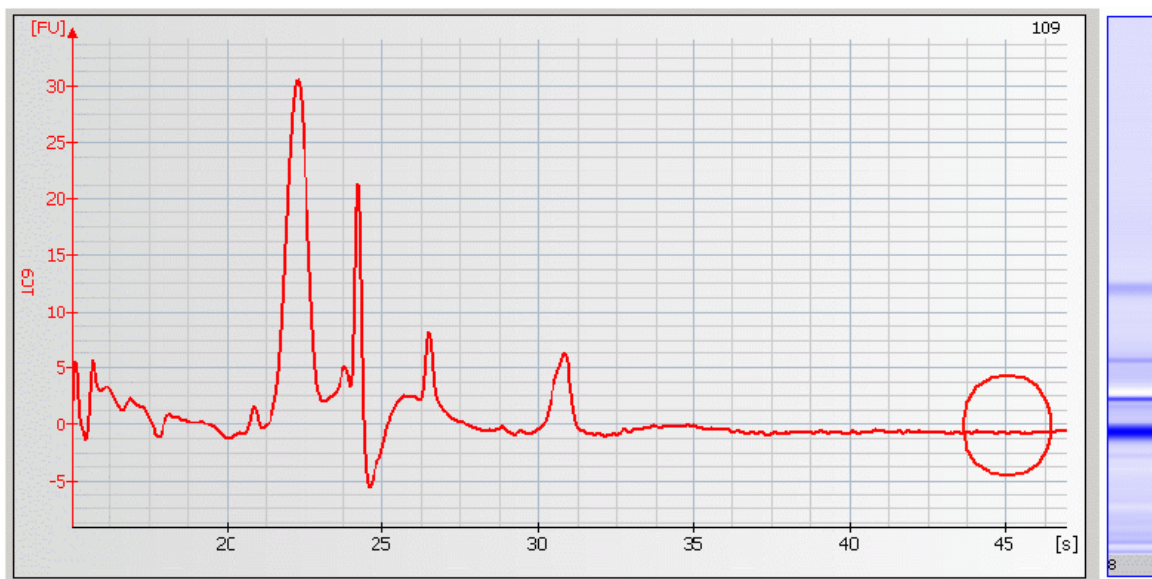


## Causes for Low or Missing Upper Marker in Ladder

<b>Most Probable Causes</b>	<b>Solution</b>
Ladder degraded.	<p>For correct ladder storage and denaturation, refer to the protein reagent kit guide. To correct for wrong selected upper marker, set upper marker manually. If necessary, adjust peak find settings. If peaks are detected that are not part of the ladder, exclude them.</p> <p>For better upper marker identification: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker. For details please refer to the Online Help or Users Guide.</p>
Diluted ladder is too old.	Use diluted ladder within one day.
<b>Probable Causes</b>	<b>Solution</b>
Ladder not denaturated properly.	Use fresh ladder aliquot. Heat ladder for 5 min at 100 °C
Ladder dried out during denaturation.	Ladder was denaturated in 1.5 ml tubes. Use 0.5 ml tubes for denaturing.

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## Low or Missing Upper Marker in Sample



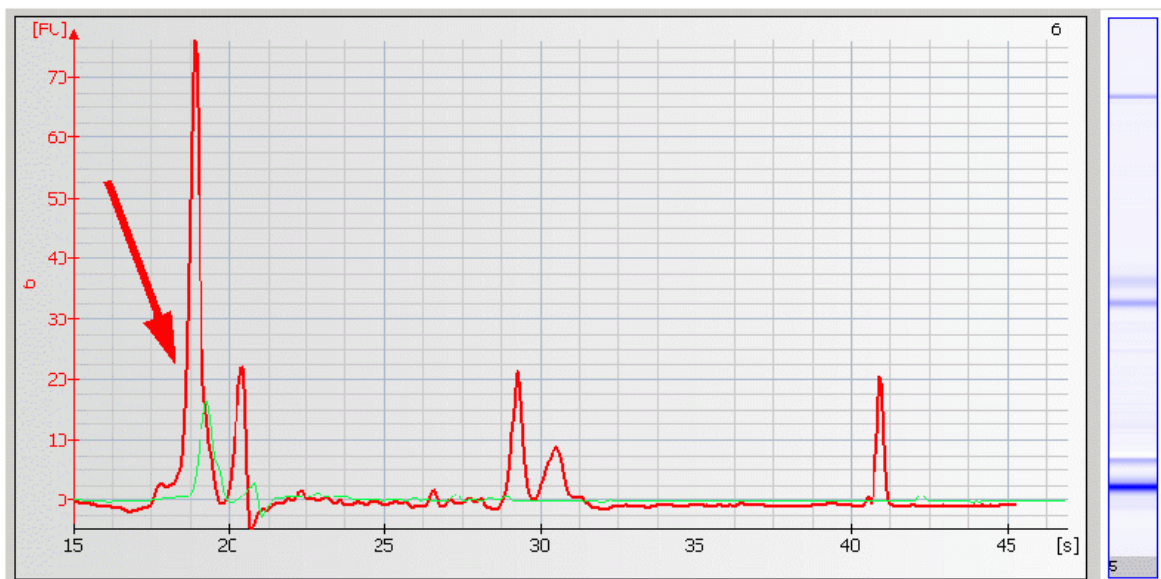
## Causes for Low or Missing Upper Marker in Sample

Most Probable Causes	Solution
Sample buffer/denaturing solution not handled according to the instructions.	<p>Refer to the instructions provided with the reagent kit guide for storage and preparation of the sample buffer/denaturing solution. To correct for wrong selected upper marker, set upper marker manually. If necessary, adjust peak find settings.</p> <p>For better upper marker identification: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the upper marker. For details please refer to the Online Help or Users Guide.</p>
Incompatible sample component. Some components of the buffer, e.g. CHAPS, TFA, etc. interfere with the upper marker and decrease sensitivity.	<p>See protein reagent kit guides for a list of compatible buffers and buffer compounds. For an updated list please refer to the web-site <a href="http://www.agilent.com/chem/labonachip">www.agilent.com/chem/labonachip</a>.</p> <p>If necessary dilute, dialyze or desalt the sample. It is recommended to dilute the samples 1:2, 1:4, ... with water to find the optimal dilution.</p>
Diluted samples are too old.	Use diluted samples within one day.

<b>Probable Causes</b>	<b>Solution</b>
Upper marker was digested by proteases.	Add protease inhibitor cocktails to cell lysate samples.
Samples not denatured properly.	Use fresh sample aliquot. Heat samples with denaturing solution for 5 min at 100 °C
Samples dried out during denaturation.	Samples were denatured in 1.5 ml tubes. Use 0.5 ml tubes for denaturing.

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## High Lower Marker Variability



### NOTE

As long as the lower marker is detected, the assay performance is not affected by lower marker or system peak variability.

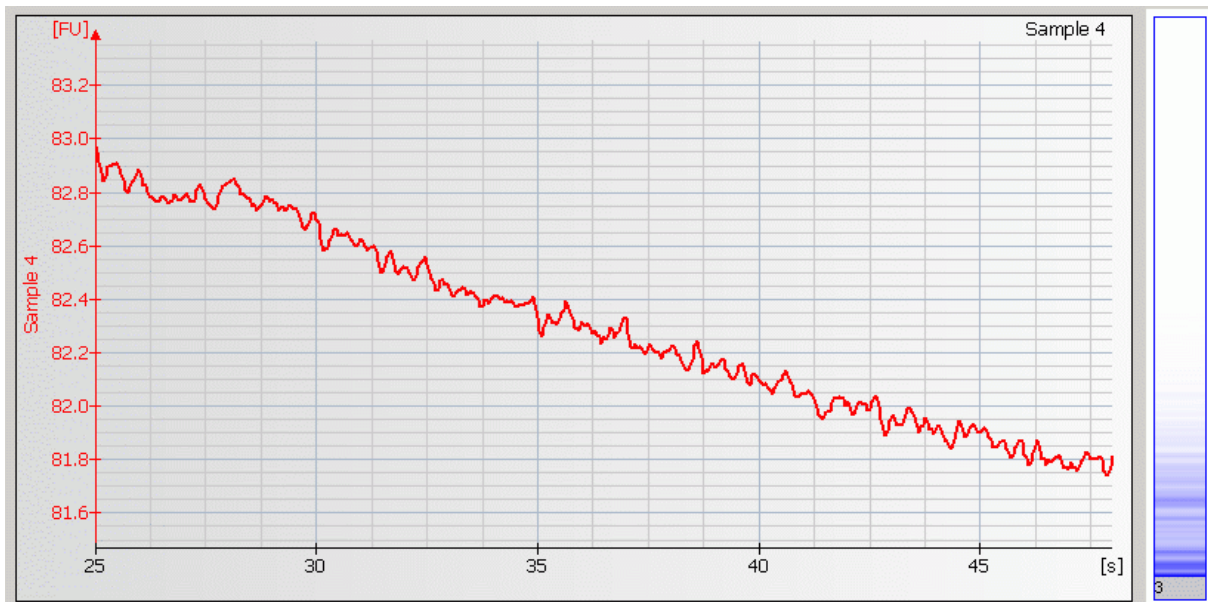


## Causes for High Lower Marker Variability

Most Probable Causes	Solution
<p>Buffer components of the sample , e.g. salts, detergents, other additives etc. interfere with the lower marker.</p> <p>Variability of ionic strength of the sample influence the lower marker intensity.</p>	<p>See protein reagent kit guide for a list of compatible buffers and buffer compounds. For an updated list please refer to the web-site <a href="http://www.agilent.com/chem/labonachip">www.agilent.com/chem/labonachip</a>.</p> <p>If necessary dilute, dialyze or desalt the sample. It is recommended to dilute the samples 1:2, 1:4, ... with water to find the optimal dilution.</p>

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# Missing Peaks

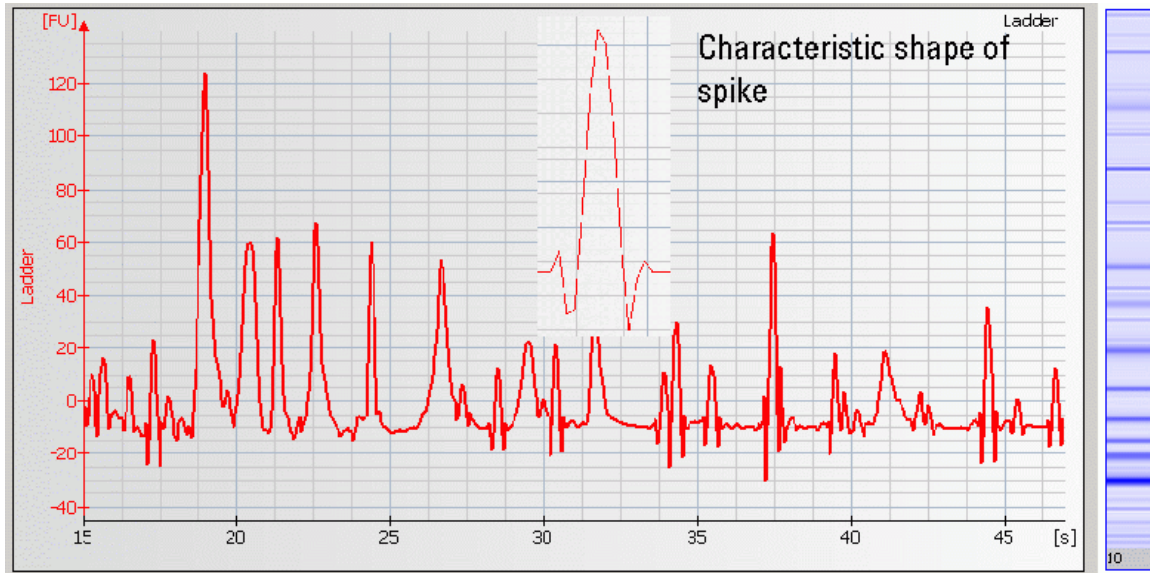


## Causes for Missing Peaks

<b>Most Probable Causes</b>	<b>Solution</b>
Laser defective.	Check laser using the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If the laser test fails, contact Agilent Technologies.
Gel dye mix was loaded in the destain well instead of destaining solution.	Discard chip and prepare new chip according to protocol.
<b>Probable Causes</b>	<b>Solution</b>
Autofocus failure.	Check autofocus using the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If autofocus test fails, contact Agilent Technologies.
<b>Least Probable Causes</b>	<b>Solution</b>
High voltage power supply defective.	Check high voltage power supply using the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If the power supply is defective, contact Agilent Technologies.
Fingerprint on focusing lens or on the backside of the chip.	Clean lens as described in “ <a href="#">Cleaning the Lens</a> ” on page 222.  Do not touch the underside of the chip

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# Spikes



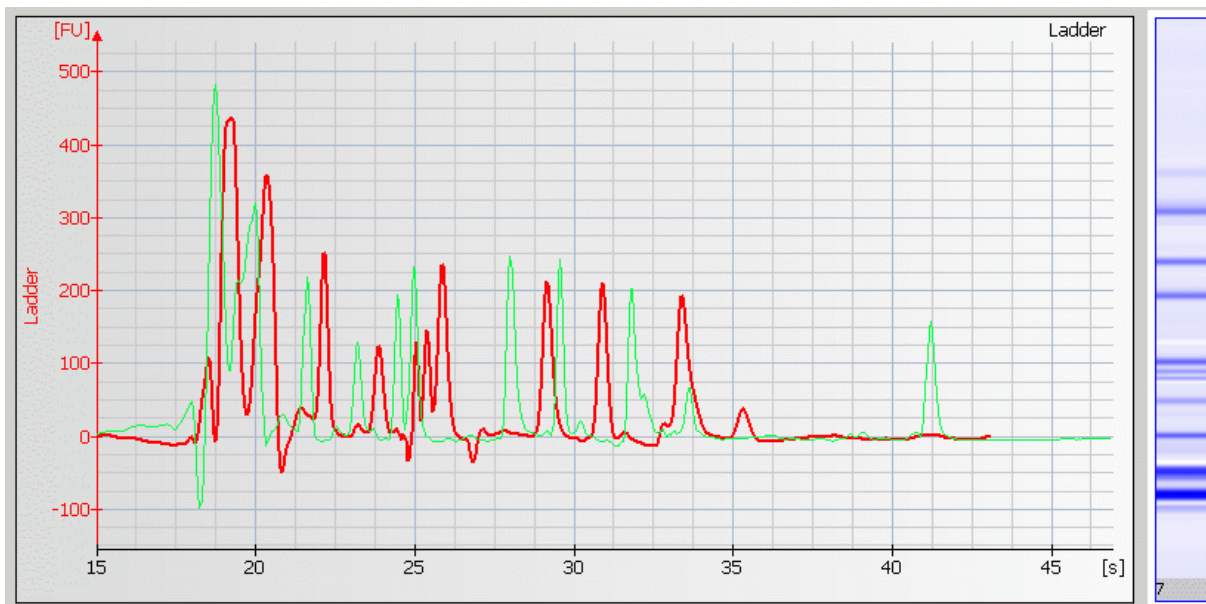
## Causes for Spikes

Most Probable Causes	Solution
<p>Chip/gel-dye mix/destaining solution/electrodes contaminated.</p>	<p>Prepare new chip with new gel-dye mix and new destaining solution:</p> <p>Wear powder-free gloves only. Don't touch the underside of the chip. Don't touch the wells of the chip.</p> <p>Clean the electrodes as described in <a href="#">"Maintenance of the Electrode Cartridge"</a> on page 180.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>
<p>Gel-dye mix/destaining solution not properly prepared.</p>	<p>Refer to the reagent kit guide for proper preparation of the gel-dye mix and destaining solution. Let the dye warm up to room temperature for 30 min before preparing the gel-dye mix. Protect the dye from light during this time</p>

Probable Causes	Solution
Vibration of Agilent 2100 bioanalyzer.	Don't touch Agilent 2100 bioanalyzer during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.

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## Poor Reproducibility



## Causes for Poor Reproducibility

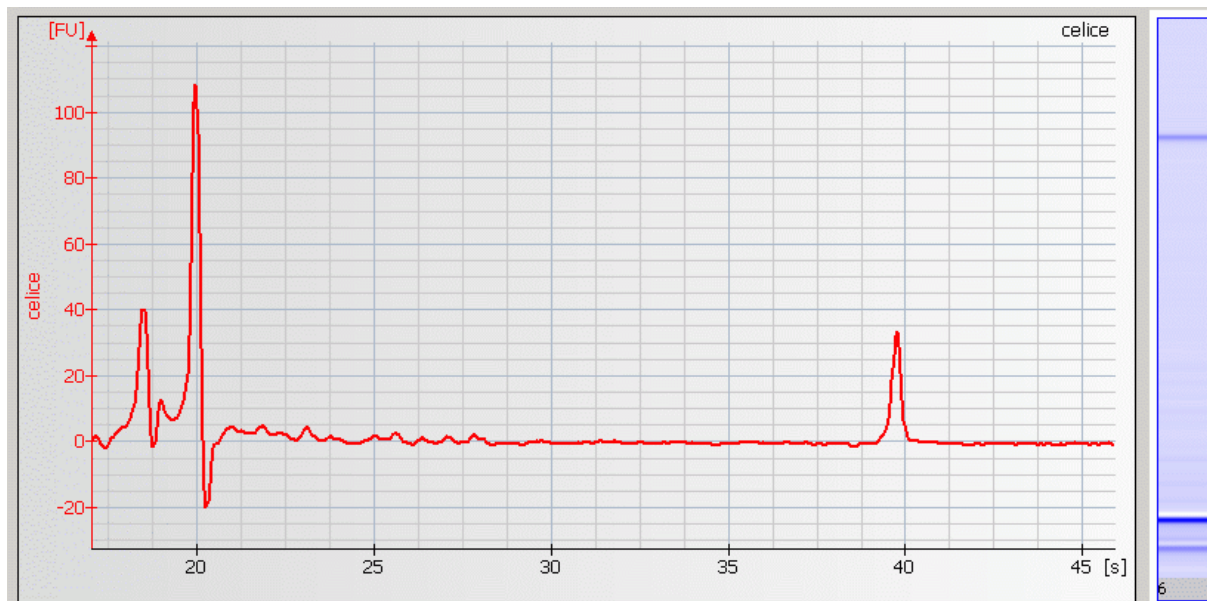
Most Probable Causes	Solution
Wrong peak alignment.	Check if alignment is correct.  For better identification of the lower and upper marker: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the lower and upper marker. For further information, please refer to the Online Help or Users Guide.
One or more samples not denaturated properly.	Use fresh sample aliquot. Heat samples with denaturing solution for 5 min at 100 °C
One or more sample dried out during denaturation.	Samples were denaturated in 1.5 ml tubes. Use 0.5 ml tubes for denaturing.
Reducing agent (BME or DTT) was added in one sample and not in the other.	Refer to the reagent kit guide for proper sample reduction.
Dirty electrodes.	Thoroughly clean the electrodes as described " <a href="#">Maintenance of the Electrode Cartridge</a> " on page 180.



Probable Causes	Solution
Diluted samples are too old.	Use diluted samples within one day.
Incompatible buffer component.	<p>See protein reagent kit guide for a list of compatible buffers and buffer compounds. For an updated list please refer to the web-site <a href="http://www.agilent.com/chem/labonachip">www.agilent.com/chem/labonachip</a>.</p> <p>If necessary dilute, dialyze or desalt the sample.</p>
Samples were not mixed properly after sample buffer and water and water was added.	Stick to the protocol as described in the reagent kit guide.
Samples were not centrifuged after denaturation	After denaturation step, spin down samples.

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## Low Sensitivity



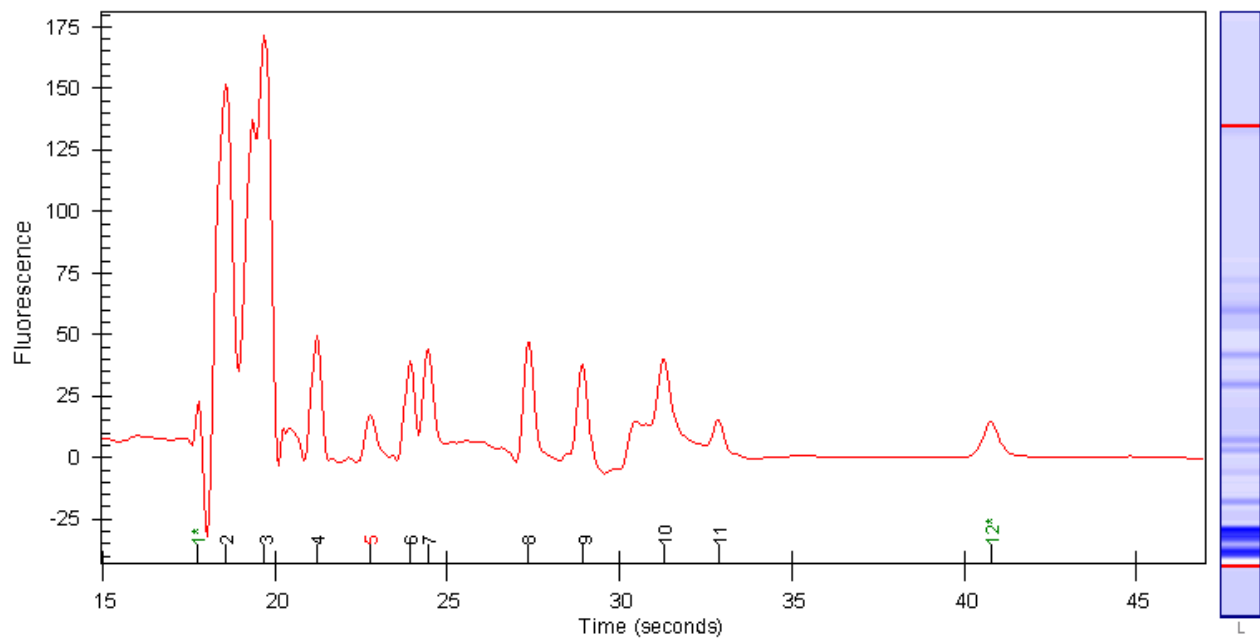
## Causes for Low Sensitivity

Most Probable Causes	Solution
Protein concentration in samples too low.	Use protein concentration according to specifications given in the reagent kit guide.
Too high salt concentration in samples.	Sensitivity is strongly affected by salt concentration. Dilute samples in deionized H <sub>2</sub> O, dialyze samples against low salt buffer or desalt samples using spin filters.
SDS not completely dissolved in dye concentrate.	Let dye concentrate equilibrate to room temperature for 30 min before use. Protect dye concentrate from light during this time. Check for undissolved SDS crystals in the tube. Vortex dye concentrate well before use. If necessary heat the sample buffer to 37 °C for 2 min.
Samples were not diluted prior to chip loading	Dilute samples according to protocol given in the reagent kit guide.

<b>Probable Causes</b>	<b>Solution</b>
Samples not completely denatured.	Use fresh sample aliquot. Heat sample/ denaturing solution for 5 min at 100 °C.
Sample/denaturing solution are dried out.	Sample/denaturing solution were denatured in 1.5 ml tubes. Use 0.5 ml tubes for denaturing.
Pipetting error during preparation of mixtures.	Check dilution procedure and check calibration of pipette.
<b>Least Probable Causes</b>	<b>Solution</b>
Samples dissolved in acidic buffer.	Neutralize samples with appropriate buffer or dilute samples in deionized H <sub>2</sub> O. Alternatively dialyze samples against buffer with medium pH.

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## Low Ladder Peaks

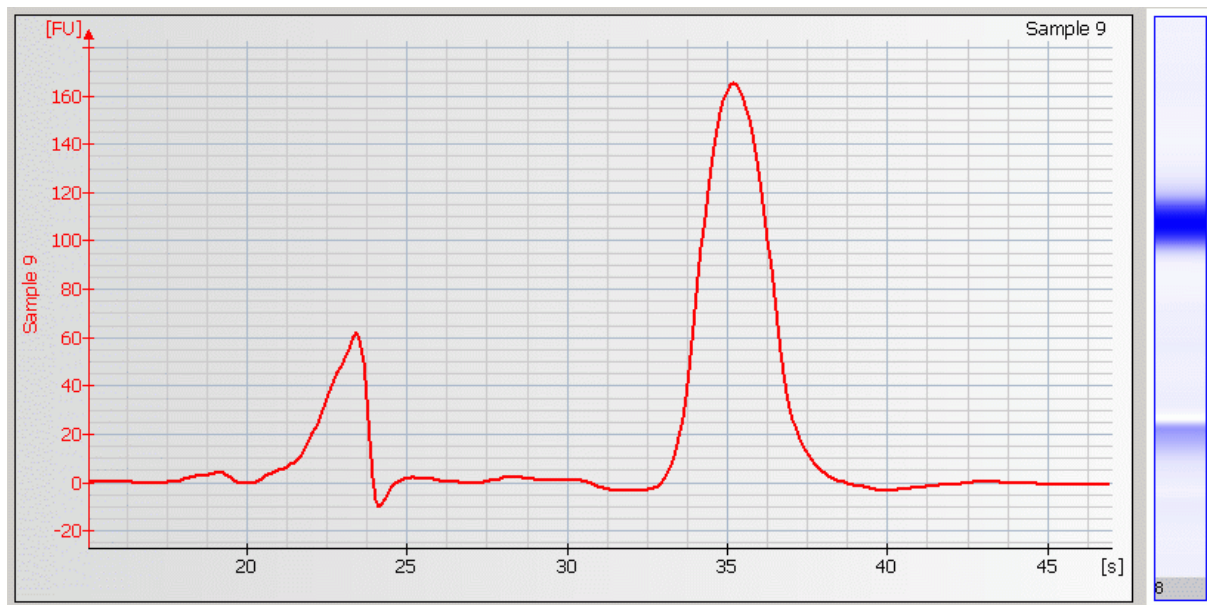


## Causes for Low Ladder Peaks

<b>Most Probable Causes</b>	<b>Solution</b>
Ladder degraded.	Refer to the protein reagent kit guide for proper ladder storage. Optional: Prepare ladder aliquots and use a new aliquot.
Ladder not diluted after denaturation.	Refer to the Reagent Kit Guide for proper chip preparation.
<b>Probable Causes</b>	<b>Solution</b>
Ladder not completely denaturated.	Use fresh ladder aliquot. Heat ladder for 5 min at 100 °C.
Ladder dried out.	Ladder was denaturated in 1.5 ml tubes. Use 0.5 ml tubes for denaturing.
Diluted ladder is too old.	Use diluted ladder within one day.
Pipetting error during preparation of mixtures.	Check dilution procedure and calibration of pipette.

Back to [“Symptoms \(Protein\)”](#) on page 121

## Broad Peaks



## Causes for Broad Peaks

Most Probable Causes	Solution
Wrong peak alignment.	<p>Check if alignment is correct (wrong alignment might cause broad peaks compared to the rest of the chip).</p> <p>For better identification of the lower and upper marker: Turn off the analysis. For correct alignment overlay electropherograms of multiple wells to clearly identify the lower and upper marker. For further information, please refer to the Online Help or Users Guide.</p>
Air bubbles in the bottom of the well.	<p>Always insert the pipette tip to the bottom of the well when dispensing the liquid. Remove large air bubbles with a pipette tip (small bubbles on top of well do not affect the assay).</p>



Chip not properly primed. Clogged chip priming station or wrong settings of priming station.

Prepare a new chip. Check chip priming station as described in “[Checking the Chip Priming Station for Proper Performance - Seal Test](#)” on page 209. Check if clip and base plate of priming station are in the correct position (see protein reagent kit guide)

Leak Current due to contaminated electrodes. Chip was left in instrument after run.

Clean electrodes with analysis-grade water and a toothbrush, see “[How to clean the Pin Set of the Electrode Cartridge](#)” on page 192. Don’t leave chip in instrument after run. Clean electrodes after each run.

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**Probable Causes****Solution**

Sample was not denaturated properly.

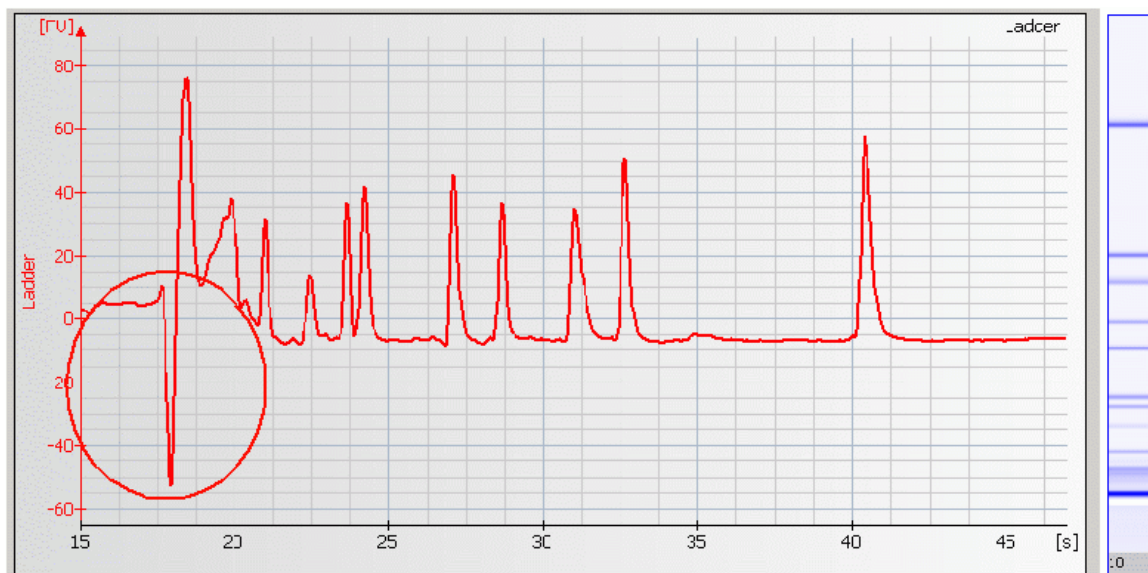
Use fresh sample aliquot. Heat sample/ denaturing solution for 5 min at 100 °C.

Reducing agent (BME or DTT) was added in one sample and not in the other.

Refer to the reagent kit guide for proper sample reduction.

Back to “[Symptoms \(Protein\)](#)” on page 121

## Baseline Dips



### NOTE

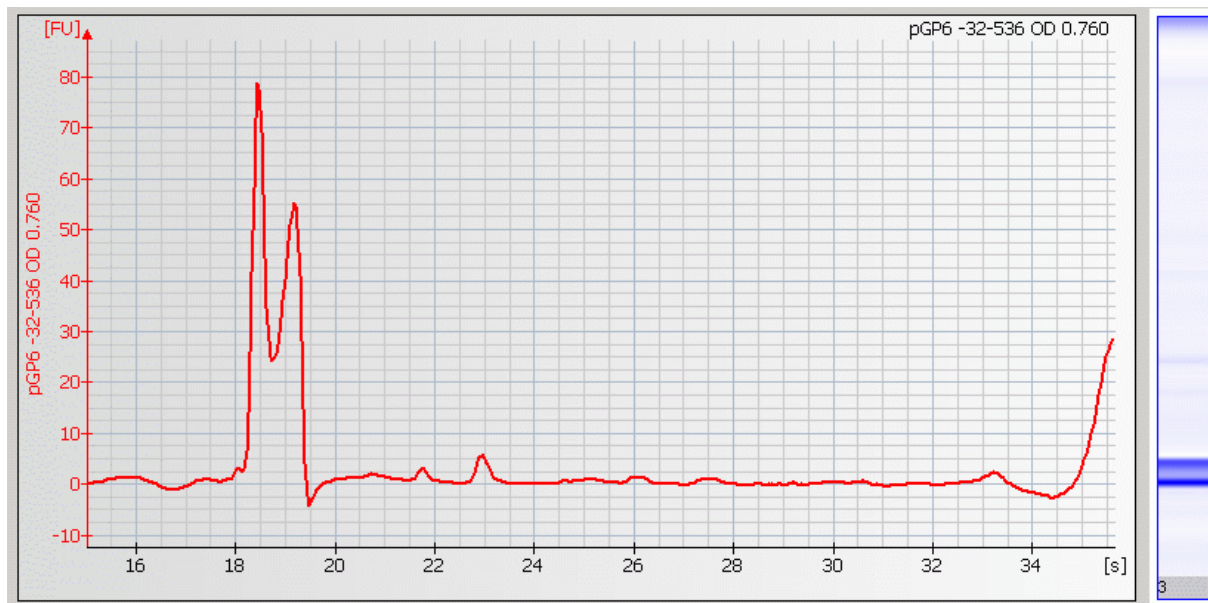
As long as the lower marker is detected, the assay performance is not affected by dips.

## Causes for Baseline Dips

Most Probable Causes	Solution
Sample contains additional detergents and/or dyes.	See protein reagent kit guide for a list of compatible buffers and buffer compounds. For an updated list please refer to the web-site <a href="http://www.agilent.com/chem/labonachip">www.agilent.com/chem/labonachip</a> .  If necessary dilute, dialyze or desalt the sample.

Back to [“Symptoms \(Protein\)”](#) on page 121

# Late Migration





## Causes for Late Migration

Most Probable Causes	Solution
Protein chips expired	Check expiration date on chip box.
Protein concentration in samples too high.	Use protein concentration according to specifications given in the reagent kit guide.
Chip not properly primed. Clogged chip priming station or wrong settings of priming station.	Prepare a new chip. Check chip priming station as described in <a href="#">“Checking the Chip Priming Station for Proper Performance - Seal Test”</a> on page 209. Clean/replace gasket and plastic adapter if necessary. Check if clip and base plate of priming station are in the correct position (see protein reagent kit guide)
Least Probable Causes	Solution
Defect Heater Plate	Run the temperature test by using the <a href="#">“Hardware Diagnostics”</a> on page 33. If the heater plate is defective, contact Agilent Technologies.

Back to [“Symptoms \(Protein\)”](#) on page 121

# Troubleshooting the Cell Application

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Click the  or  button next to the error message to view a help screen that is specific for that error.

Additional information regarding the nature of a problem can often be found in the Run Log of the data file. Select the Log Book tab in the Data and Assay context. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by your Agilent 2100 bioanalyzer might not be what you expected. To help you find the reason for the discrepancy, see “[Symptoms \(Cell\)](#)” on page 168.

For most observations you will find at least one corresponding example, depicting a typical histogram. Once you have identified the observation that resembles the outcome of your experiment, you will get a set of assigned causes listed by probability.

The causes are grouped into three levels:

- most probable cause
- probable cause
- least probable cause

A list of solutions that help you to fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by priority.

## Symptoms (Cell)

Click the icon to see an example, or go straight to the troubleshooting hints.

- [“Clogged Pressure Adapter”](#) on page 169
- [“No Cell Events”](#) on page 170
- [“Low Cell Events”](#) on page 173
- [“Low Sensitivity”](#) on page 176
- [“High Events”](#) on page 178



## Clogged Pressure Adapter

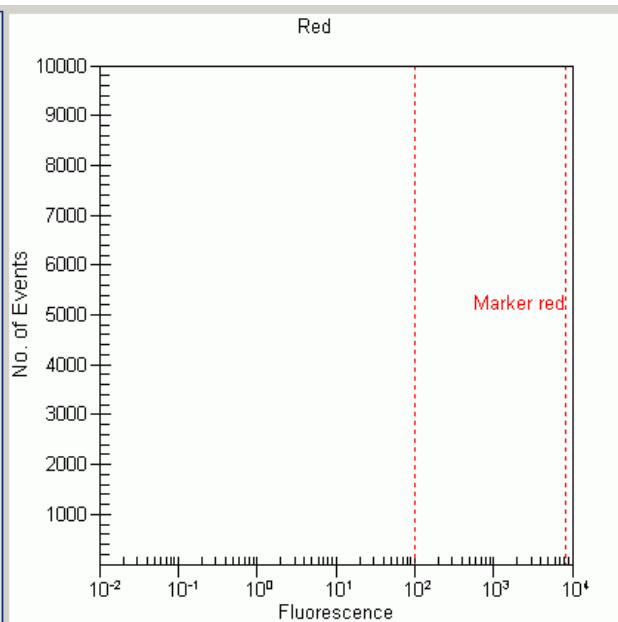
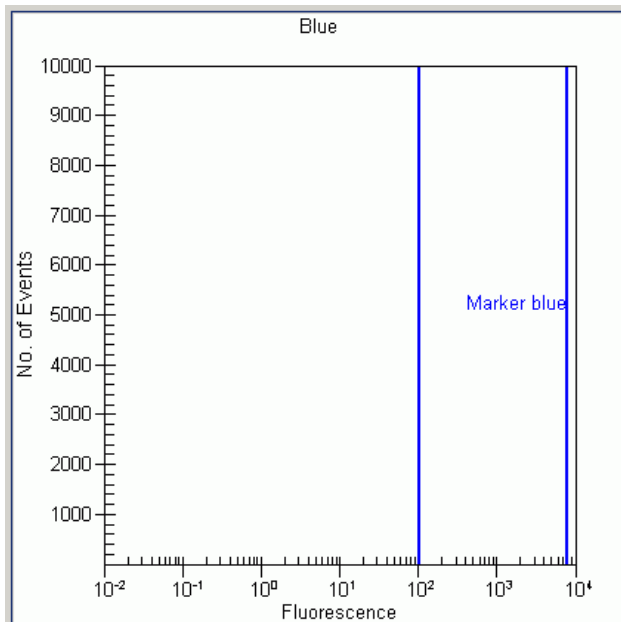
Most Probable Causes	Solution
Liquid is drawn from the priming well into the adapter, because no or not enough buffer was filled in the buffer well.	Replace the pressure adapter as described in <a href="#">“Replacing the Pressure Adapter”</a> on page 216.  Prepare a new chip: Refer to the Cell Reagent Kit Guide for proper chip preparation. Do not leave any wells empty.
Liquid is drawn from the priming well into the adapter, because no or not enough focusing dye/sample was filled in focusing dye/sample well.	Replace the pressure adapter as described in <a href="#">“Replacing the Pressure Adapter”</a> on page 216.  Prepare a new chip: Refer to the Cell Reagent Kit Guide for proper chip preparation. Do not leave any wells empty.

Back to [“Symptoms \(Cell\)”](#) on page 168

# No Cell Events

Links to Examples:

Context ID:



	Marker	Min	Max	#Events	%Total	% of gated
▶	● All Events	0	0	0	0	0

	Marker	Min	Max	#Events	%Total	% of gated

## Causes for No Cell Events

<b>Most Probable Causes</b>	<b>Solution</b>
No cells in sample.	Prepare a new chip: Use cell concentration as given in the Reagent Kit guide. Check cell concentration with a counting chamber. Adjust concentration if necessary. Visually inspect sample well under microscope to confirm that cells were loaded correctly.
Bead sample not prepared according to the instructions.	Prepare a new chip: Please refer to the Cell Fluorescence Checkout Kit Guide for proper bead preparation.
Low staining efficiency.	Check staining procedure. Always prepare positive and negative staining control. Your staining protocol (dye concentration, incubation time, temperature,...) may need optimization for your specific cell preparation. For application specific protocols and recommended staining reagents please refer to available application notes ( <a href="http://www.agilent.com/chem/labonachip">http://www.agilent.com/chem/labonachip</a> ).

<b>Probable Causes</b>	<b>Solution</b>
Cells not resuspended in CB.	Always resuspend cells in CB at an appropriate cell concentration before analysis.
Used dye not compatible with bioanalyzer optics.	For application specific protocols and recommended staining reagents please refer to available application notes ( <a href="http://www.agilent.com/chem/labonachip">http://www.agilent.com/chem/labonachip</a> ).

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Not enough buffer or focusing dye in chip wells.	Prepare a new chip: Refer to the Cell Reagent Kit Guide for proper chip preparation. Do not leave any wells empty.
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Chip not properly primed. Unremovable air bubble in chip.	Prepare a new chip: Prime the chip according to the “ <a href="#">Essential Measurement Practices</a> ” on page 10.
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Chip channel clogged.	Prepare a new chip: Use cell strainer for clumpy cell sample.
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### **Least Probable Causes**

### **Solution**

Pipetting error during cell preparation.	Use appropriate pipettes and pipette tips. Check calibration of pipette.
--	--

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Autofocus failure.	Check autofocus using the “ <a href="#">Hardware Diagnostics</a> ” on page 33.
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If Optical Drive test fails, contact Agilent Technologies.

---

Chip contaminated.
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Prepare a new chip:

Wear powder-free gloves only.

Do not touch the underside of the chip.

Do not touch the wells of the chip.

Load the chip immediately after taking it out of its sealed bag.

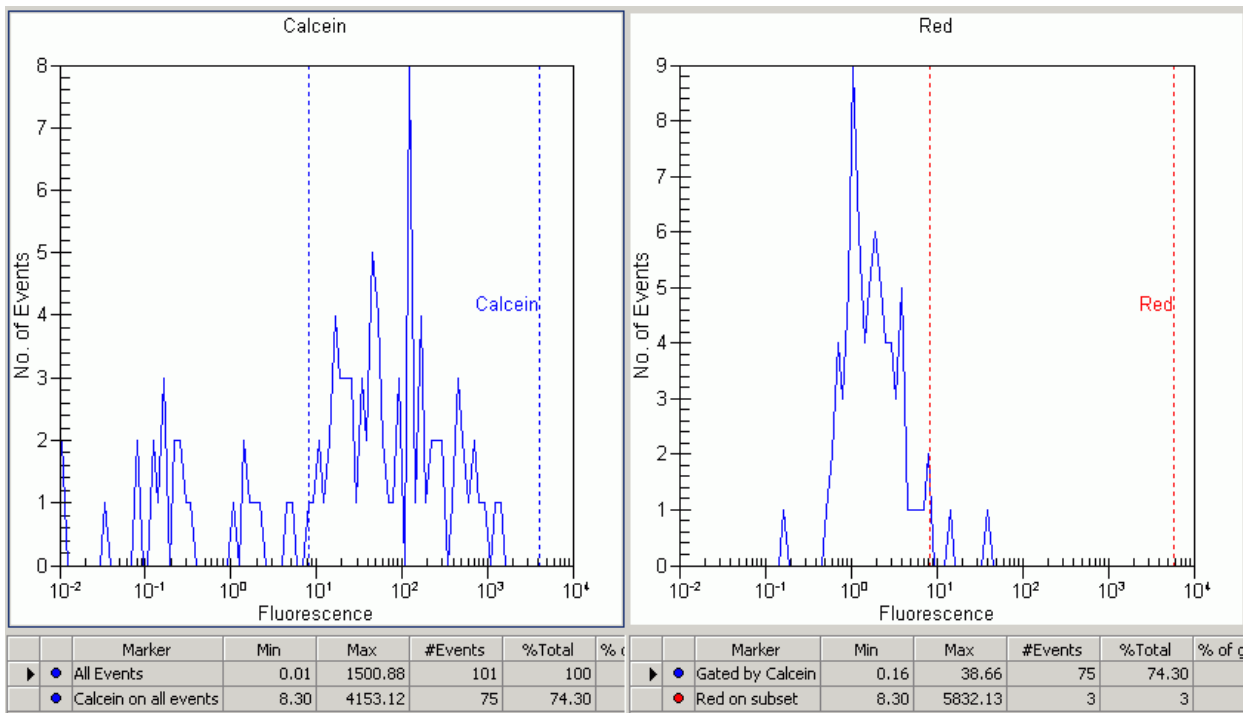
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Back to “[Symptoms \(Cell\)](#)” on page 168

# Low Cell Events

Links to Examples:

Context ID:



## NOTE

At the recommended cell concentration of 2 million/ml, an average of 750 cells are measured per sample. Event numbers below 400 are considered as low.

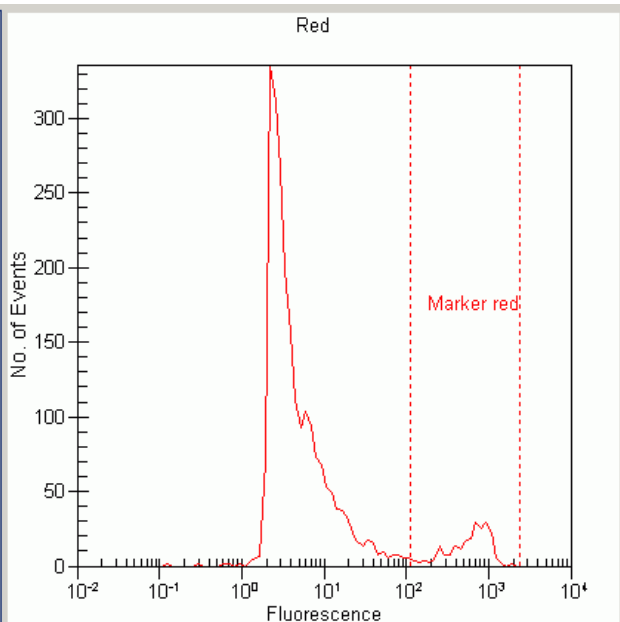
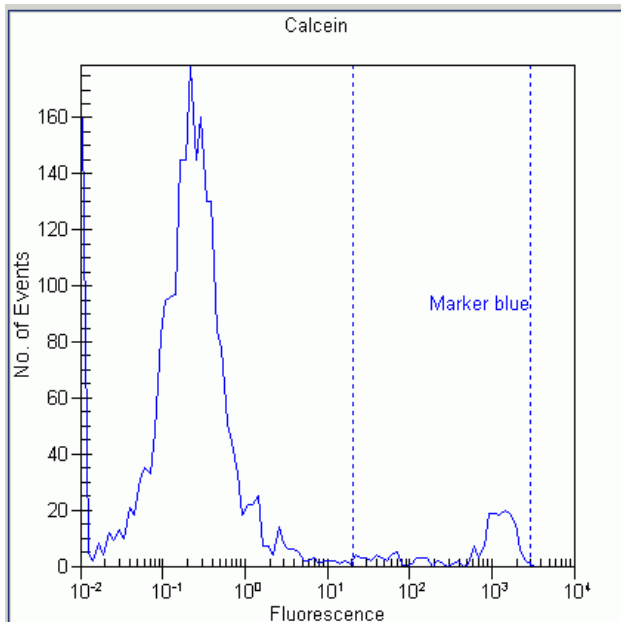
## Causes for Low Cell Events

<b>Most Probable Causes</b>	<b>Solution</b>
Low cell concentration.	Prepare a new chip: Use cell concentration of 2 million cells/ml as recommended in the Reagent Kit guide. Check cell concentration with a counting chamber. Adjust concentration if necessary.
Bead sample not prepared according to the instructions.	Prepare a new chip: Please refer to the Cell Fluorescence Checkout Kit Guide for proper bead preparation.
Low staining efficiency.	Check staining procedure. Always prepare positive and negative staining control. Your staining protocol (dye concentration, incubation time, temperature,...) may need optimization for your specific cell preparation. For application specific protocols and recommended staining reagents please refer to available application notes ( <a href="http://www.agilent.com/chem/labonachip">http://www.agilent.com/chem/labonachip</a> ).
<b>Probable Causes</b>	<b>Solution</b>
Not enough buffer in buffer well.	Prepare a new chip: Refer to the Cell Reagent Kit Guide for proper chip preparation.
No focusing dye in FD-well.	Prepare a new chip: Refer to the Cell Reagent Kit Guide for proper chip preparation.
Not enough sample in sample well.	Prepare a new chip: Refer to the Cell Reagent Kit Guide for proper chip preparation.

Chip not properly primed. Unremovable air bubble in chip.	Prepare a new chip: Prime the chip according to the “ <a href="#">Essential Measurement Practices</a> ” on page 10.
Chip channel clogged.	Prepare a new chip: Use cell strainer for clumpy cell sample.
<b>Least Probable Causes</b>	<b>Solution</b>
Pipetting error during cell preparation.	Use appropriate pipettes and pipette tips. Check calibration of pipette.
Autofocus failure.	Check autofocus using the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If Optical Drive test fails, contact Agilent Technologies.
Chip contaminated.	<p>Prepare a new chip:</p> <p>Wear powder-free gloves only.</p> <p>Do not touch the underside of the chip.</p> <p>Do not touch the wells of the chip.</p> <p>Load the chip immediately after taking it out of its sealed bag.</p>

Back to “[Symptoms \(Cell\)](#)” on page 168

# Low Sensitivity



	Marker	Min	Max	#Events	%Total
▶	● All Events	0.01	2829.89	2450	100
	● Marker blue on all events	20.20	2891.20	185	7.60

	Marker	Min	Max	#Events	%Total
▶	● All Events	0.13	1915.89	2450	100
	● Marker red on all events	110.13	2351.03	212	8.70



## Causes for Poor Fluorescence Intensity

Most Probable Causes	Solution
Low staining efficiency.	Check staining procedure. Always prepare positive and negative staining control. The staining protocol (dye concentration, incubation time, temperature,...) may need optimization for your specific cell preparation. For application specific protocols and recommended staining reagents please refer to available application notes ( <a href="http://www.agilent.com/chem/labonachip">http://www.agilent.com/chem/labonachip</a> ).
Least Probable Causes	Solution
Autofocus failure.	Check autofocus using the “ <a href="#">Hardware Diagnostics</a> ” on page 33. If Optical Drive test fails, contact Agilent Technologies.
Poor focusing because of decomposed focusing dye.	Prepare a new chip: Use fresh focusing dye. Protect the focusing dye solution from light.

Back to “[Symptoms \(Cell\)](#)” on page 168

## High Events

	Sample Name	Sample Comment	Blue Staining	Red Staining	Status	Total Events	% of Gated	Obse
▶	J 0.2uM		Oreg Green	CBNF	✓	5220	N/A	
2	J 1uM		Oreg Green	CBNF	✓	5130	N/A	
3	J 5uM		Oreg Green	CBNF	✓	4435	N/A	
4	J 25uM		Oreg Green	CBNF	✓	2651	N/A	
5	Sample 5		Oreg Green	CBNF	✓	0	0	
6	Sample 6		Oreg Green	CBNF	✓	0	0	

## Causes for High Event Numbers

<b>Most Probable Causes</b>	<b>Solution</b>
Too high cell concentration.	Results may be inaccurate. Consider to prepare a new chip: Use cell concentration as given in the Reagent Kit guide. Check cell concentration with a counting chamber. Adjust concentration if necessary.
Bead sample not prepared according to the instructions.	Prepare a new chip: Please refer to the Cell Fluorescence Checkout Kit Guide for proper bead preparation.
<b>Probable Causes</b>	<b>Solution</b>
Wrong assay selected: a conventional assay was selected, while the staining was performed on-chip	Import markers and settings from the corresponding on-chip assay.
Not enough buffer in buffer well.	Prepare a new chip: Refer to the Cell Reagent Kit Guide for proper chip preparation.
Not enough sample in sample well.	Prepare a new chip: Refer to the Cell Reagent Kit Guide for proper chip preparation.

Back to [“Symptoms \(Cell\)”](#) on page 168

# Maintenance of the Electrode Cartridge

## Overview

The cleaning procedure of the electrode cartridge (re-order no. 5065-4413) depends on the assay that is run on the 2100 bioanalyzer. The following table gives an overview on the different cleaning procedures:

Assay	Before each Run	After each Run	Monthly or after Liquid Spill: Pin Set Cleaning
DNA and Protein Assays	not required	electrode cleaner: deionized H <sub>2</sub> O (10 s)	with brush: deionized H <sub>2</sub> O or isopropanol  For details see <a href="#">“How to clean the Pin Set of the Electrode Cartridge”</a> on page 192.

<b>Assay</b>	<b>Before each Run</b>	<b>After each Run</b>	<b>Monthly or after Liquid Spill: Pin Set Cleaning</b>
RNA Nano Assay	electrode cleaner: first RNaseZAP (60 s), followed by RNase free H <sub>2</sub> O (10 s)	electrode cleaner: RNase free H <sub>2</sub> O (10 s)	RNase decontamination: with brush: first RNaseZAP followed by RNase free H <sub>2</sub> O  For details see “ <a href="#">How to clean the Pin Set of the Electrode Cartridge</a> ” on page 192.
RNA Pico Assay	electrode cleaner: RNase free H <sub>2</sub> O (5 min)	electrode cleaner: RNase free H <sub>2</sub> O (30 s)	RNase decontamination: with brush: first RNaseZAP followed by RNase free H <sub>2</sub> O  For details see “ <a href="#">How to clean the Pin Set of the Electrode Cartridge</a> ” on page 192.

## DNA and Protein Assays

When the assay is complete, immediately remove the used chip out of the Agilent 2100 bioanalyzer and dispose of it according to good laboratory practice. Then perform the following procedure to ensure that the electrodes are clean (no residues are left over from the previous assay).

### When required

- after each DNA and Protein run

### Material required

- Electrode Cleaner (included in the Protein and DNA kits)
- deionized analysis-grade water

### Procedure

- 1 Slowly fill one of the wells of the electrode cleaner with 350  $\mu$ l deionized analysis-grade water.

---

#### CAUTION

Never fill too much water in the electrode cleaner. This could cause liquid spill which might cause leak currents between the electrodes.

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- 2 Open the lid and place electrode cleaner in the Agilent 2100 bioanalyzer.
- 3 Close the lid and leave it closed for about 10 seconds.
- 4 Open the lid and remove the electrode cleaner.
- 5 Wait another 10 seconds for the water on the electrodes to evaporate.

- 6 After 5 assays, empty and refill the electrode cleaner
- 7 After 25 assays, replace the used electrode cleaner by a new one.

---

NOTE

When switching between different assays, a more thorough cleaning may be required. For details, refer to “[How to clean the Pin Set of the Electrode Cartridge](#)” on page 192.

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# RNA Nano Assay

Assay	Before each Run	After each Run	Monthly or after Liquid Spill: Pin Set Cleaning
RNA Nano	electrode cleaner: first RNaseZAP (60 s), followed by RNase free H <sub>2</sub> O (10 s)	electrode cleaner: RNase free H <sub>2</sub> O (10 s)	RNase decontamination: with brush: first RNaseZAP followed by RNase free H <sub>2</sub> O

## Cleaning the Electrodes before each RNA Nano Assay

To avoid decomposition of your RNA sample, follow this electrode decontamination procedure on a daily basis before running any RNA Nano assay.

### When required

- before each RNA Nano run

### Material required

- 2 Electrode Cleaners (included in the RNA 6000 Nano kit)
- RNase-free water
- RNaseZAP (Ambion, Inc cat. no. 9780)



## Procedure

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### NOTE

Perform the following RNase decontamination procedure on a daily basis before running any RNA Nano assays.

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- 1 Slowly fill one of the wells of an electrode cleaner with 350  $\mu$ l RNaseZAP.
  - 2 Open the lid and place electrode cleaner in the Agilent 2100 bioanalyzer.
  - 3 Close the lid and leave it closed for about 1 minute.
  - 4 Open the lid and remove the electrode cleaner—label the electrode cleaner and keep for future use. You can reuse the electrode cleaner for all the chips in the kit.
  - 5 Slowly fill one of the wells of *another* electrode cleaner with 350  $\mu$ l RNase-free water.
  - 6 Place electrode cleaner in the Agilent 2100 bioanalyzer.
  - 7 Close the lid and leave it closed for about 10 seconds.
  - 8 Open the lid and remove the electrode cleaner- label it and keep it for further use.
  - 9 Wait another 10 seconds for the water on the electrodes to evaporate before closing the lid.
- 

### NOTE

Remove the RNase ZAP and the RNase-free water from the electrode cleaner at the end of the day.

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## Cleaning the Electrodes after each RNA Nano Assay

When the assay is completed, immediately remove the used chip from the Agilent 2100 bioanalyzer and dispose of it according to good laboratory practice. Then perform the following procedure to ensure that the electrodes are clean (no residues are left over from the previous assay).

**When required**

- after each RNA Nano run

**Material required**

- Electrode Cleaner (included in the RNA 6000 Nano kit)
- RNase-free water

### Procedure

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#### NOTE

Use a new electrode cleaner with each new kit.

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**1** Slowly fill one of the wells of the electrode cleaner with 350  $\mu$ l RNase free water.

---

#### CAUTION

Never fill too much water in the electrode cleaner. This could cause liquid spill which might cause leak currents between the electrodes.

---

**2** Open the lid and place electrode cleaner in the Agilent 2100 bioanalyzer.

**3** Close the lid and leave it closed for about 10 seconds.

- 4 Open the lid and remove the electrode cleaner.
- 5 Wait another 10 seconds for the water on the electrodes to evaporate.

---

#### NOTE

Remove the RNase-free water from the electrode cleaner at the end of the day.

---

### **RNase Decontamination of the Pin Set**

When the pin set of the electrode cartridge is suspected to be contaminated with RNases follow the instructions described in “[How to clean the Pin Set of the Electrode Cartridge](#)” on page 192.

# RNA Pico Assay

Assay	Before each Run	After each Run	Monthly or after Liquid Spill: Pin Set Cleaning
RNA Pico	electrode cleaner: RNase free H <sub>2</sub> O (5 min)	electrode cleaner: RNase free H <sub>2</sub> O (30 s)	RNase decontamination: with brush: first RNaseZAP followed by RNase free H <sub>2</sub> O

## Cleaning the Electrodes before each RNA Pico Assay

To avoid decomposition of your RNA sample, follow this electrode decontamination procedure on a daily basis before running any RNA Pico assay.

### When required

- before each RNA Pico run

### Material required

- Electrode Cleaners (3 electrode cleaners are included in the RNA 6000 Pico kit)
- RNase-free water

## Procedure

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### NOTE

To prevent contamination problems, it is strongly recommended to use a dedicated electrode cartridge for RNA assays. For running the RNA 6000 Pico assay, the 16 pin-bayonet electrode cartridge is mandatory.

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- 1 Slowly fill one of the wells of an electrode cleaner with 350  $\mu$ l RNase free water.
- 2 Open the lid and place electrode cleaner in the Agilent 2100 bioanalyzer.
- 3 Close the lid and leave it closed for 5 minutes.
- 4 Open the lid and remove the electrode cleaner—label the electrode cleaner and keep for future use.
- 5 Wait another 30 seconds for the water on the electrodes to evaporate before closing the lid.

## Cleaning the Electrodes after each RNA Pico Assay

When the assay is complete, immediately remove the used chip out of the Agilent 2100 bioanalyzer and dispose of it according to good laboratory practice. Then perform the following procedure to ensure that the electrodes are clean (no residues are left over from the previous assay).

**When required**

- after each RNA Pico run

**Material required**

- Electrode Cleaners (3 electrode cleaners are included in the RNA 6000 Pico kit)
- RNase-free water

### Procedure

1 Slowly fill one of the wells of an electrode cleaner with 350  $\mu$ l RNase free water.

---

#### CAUTION

Never fill too much water in the electrode cleaner. This could cause liquid spill which might cause leak currents between the electrodes.

---

2 Open the lid and place electrode cleaner in the Agilent 2100 bioanalyzer.

3 Close the lid and leave it closed for 30 seconds.

4 Open the lid and remove the electrode cleaner.

- 5 Wait another 30 seconds for the water on the electrodes to evaporate before closing the lid.

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#### NOTE

Replace the water in the electrode cleaner after *each* use. Use a new electrode cleaner after 12-13 electrode cleaning procedures and with new kit.

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## **RNase Decontamination of the Pin Set**

When the pin set of the electrode cartridge is suspected to be contaminated with RNases follow the instructions described in “[How to clean the Pin Set of the Electrode Cartridge](#)” on page 192.

## How to clean the Pin Set of the Electrode Cartridge

The electrode cartridge, which includes the pin set, can be removed if the electrodes become contaminated.

### When required

- on a monthly basis
- whenever the pin set is contaminated with liquid spill or salt deposition
- when the pin set is contaminated with RNases

### Material required

- deionized analysis grade water (DNA and Protein Assay) or RNase-free water (RNA Assays)
- in case of RNase contamination: RNaseZAP (Ambion, Inc cat. no. 9780)
- unused chip to run the short circuit diagnostic test

### Tools required

- compressed oil-free air or desiccator
- beaker
- soft brush

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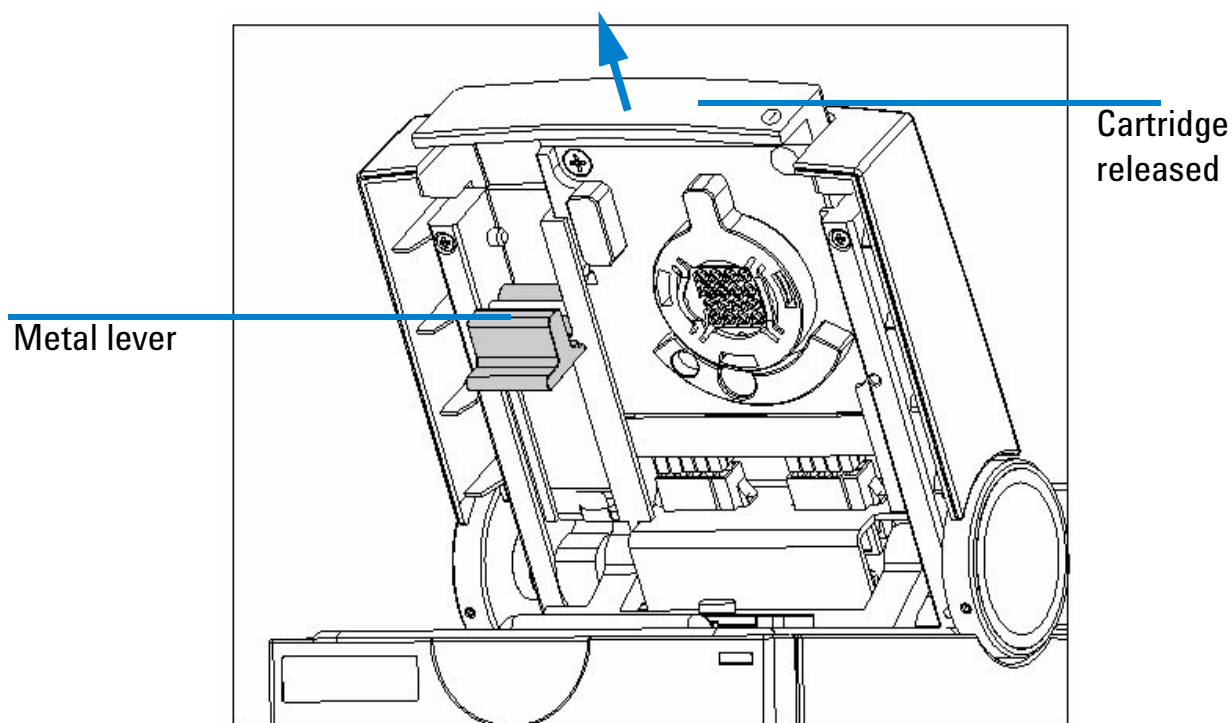
### CAUTION

Do not touch the electrodes while the cartridge is in the 2100 bioanalyzer—this could cause damage to the electrodes and high voltage power supplies.

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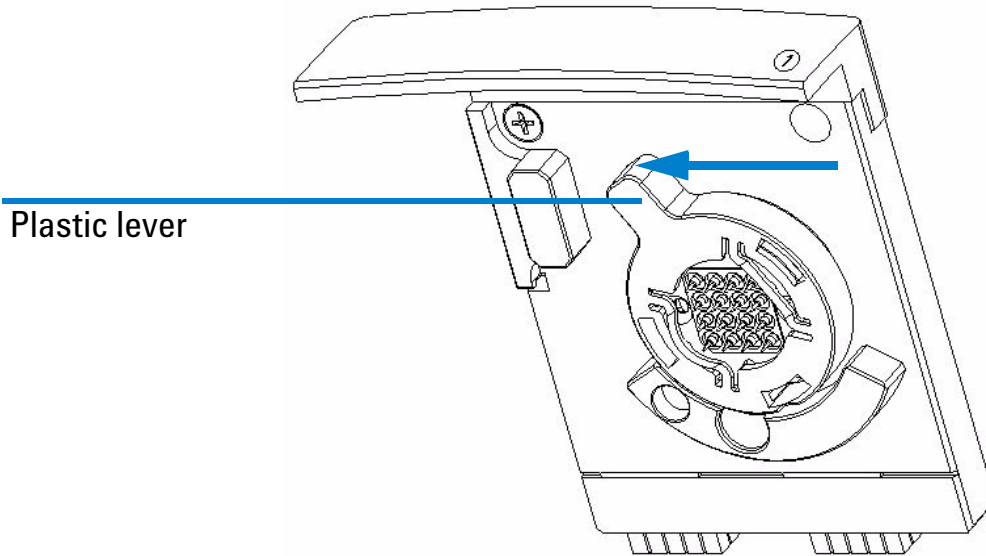


- 1 Turn off line power to the 2100 bioanalyzer. The line switch is located at the rear of the 2100 bioanalyzer.
- 2 Open the lid and pull the metal lever on the inside left of the lid to the vertical position as shown in Figure 2. When the lever is in the vertical position, the cartridge is released from the lid by about 10 mm.
- 3 Gently pull the cartridge out of the lid as shown in Figure 3, “[Removing the Electrode Cartridge](#)”.



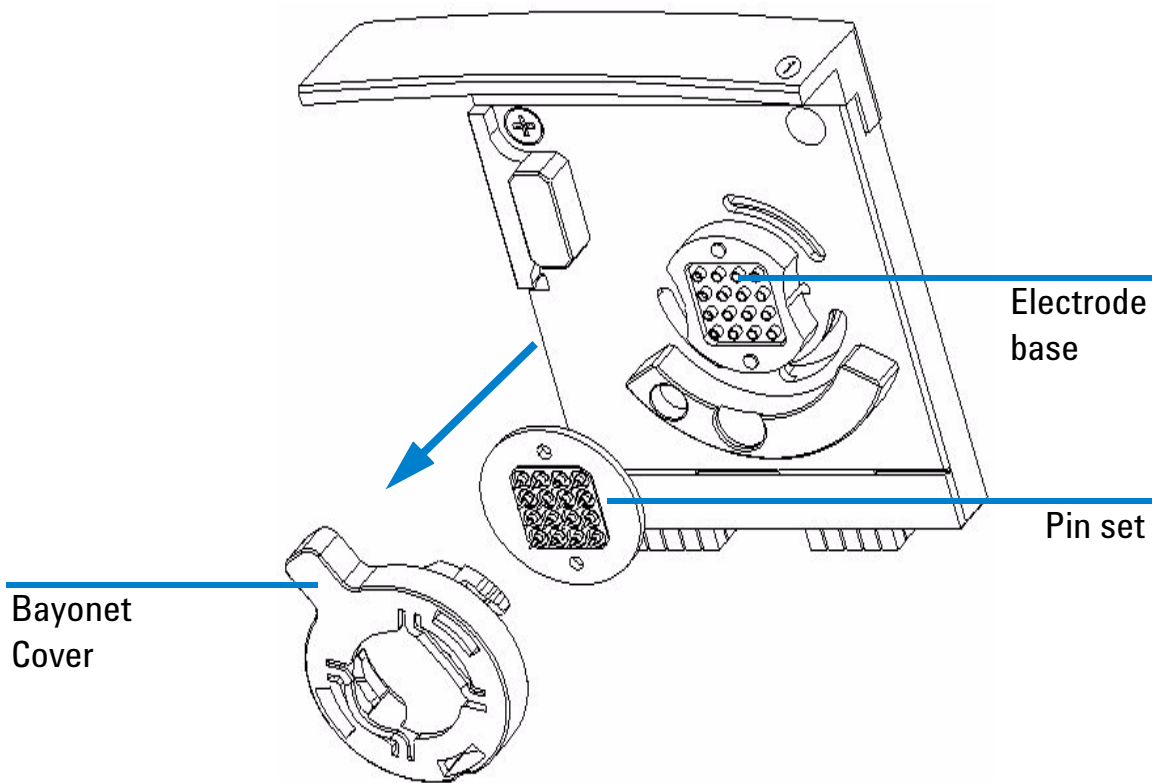
**Figure 3 Removing the Electrode Cartridge**

- 4 Open the bayonet socket of the pin set by turning the plastic lever to the left as described in Figure 4, “Opening the bayonet socket of the Pin Set”.



**Figure 4** Opening the bayonet socket of the Pin Set

- 5 Remove the cover of the bayonet socket by gently pulling the plastic lever. The pin set may stick to the electrode base. Remove it by carefully pulling it off. See Figure 5, “Releasing the Pin Set”.



**Figure 5 Releasing the Pin Set**

- 6 Gently brush the pin set with a soft brush in deionized analysis-grade water or isopropanol. In case of RNase contamination use RNase Zap (Ambion, Inc cat. no. 9780).
- 

### CAUTION

Be careful not to bent or misalign the pins. Both will lead to poor quality results or pre-terminated assay runs.

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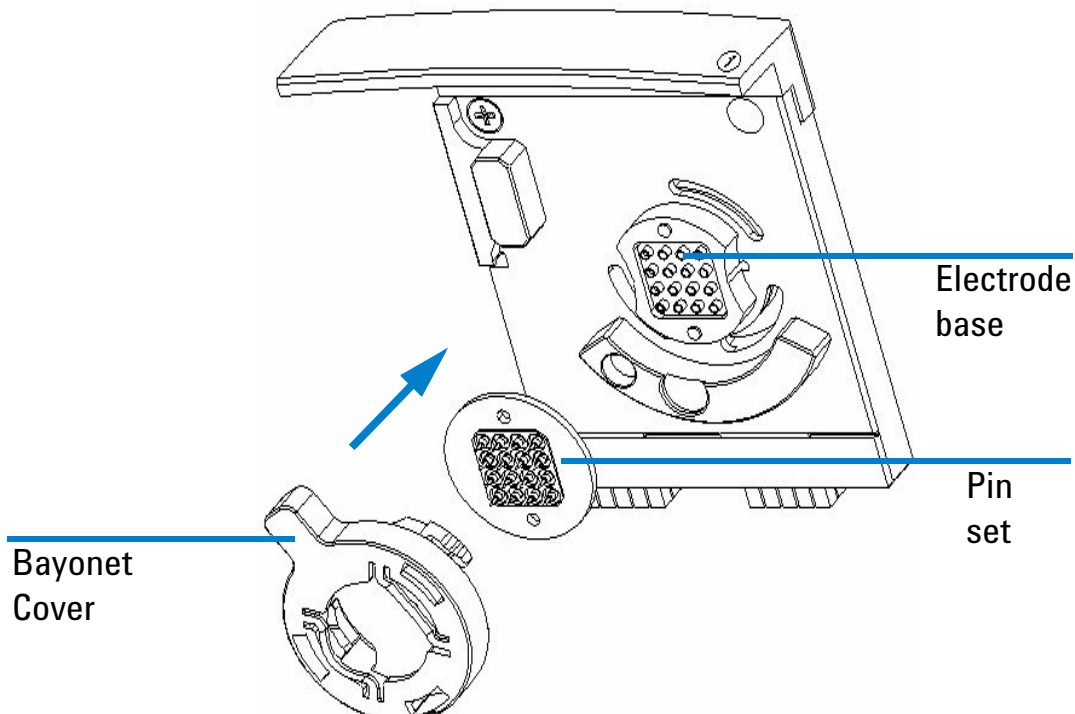
- 7 In case of highly contaminated or dirty pins you may autoclave or sonicate the pin set. For autoclaving or sonicating the pin set, follow your standard procedures for plastic material.
  - 8 Rinse pin set thoroughly with deionized analysis-grade water when running DNA or Protein assays or RNase-free water when running RNA assays.
  - 9 Let the pin set completely dry in a desiccator overnight or use oil-free compressed air.
- 

### CAUTION

Make sure that the pin set is completely dry before placing it back into the electrode base. Even small amounts of liquid on the pin set can damage the high voltage power supply.

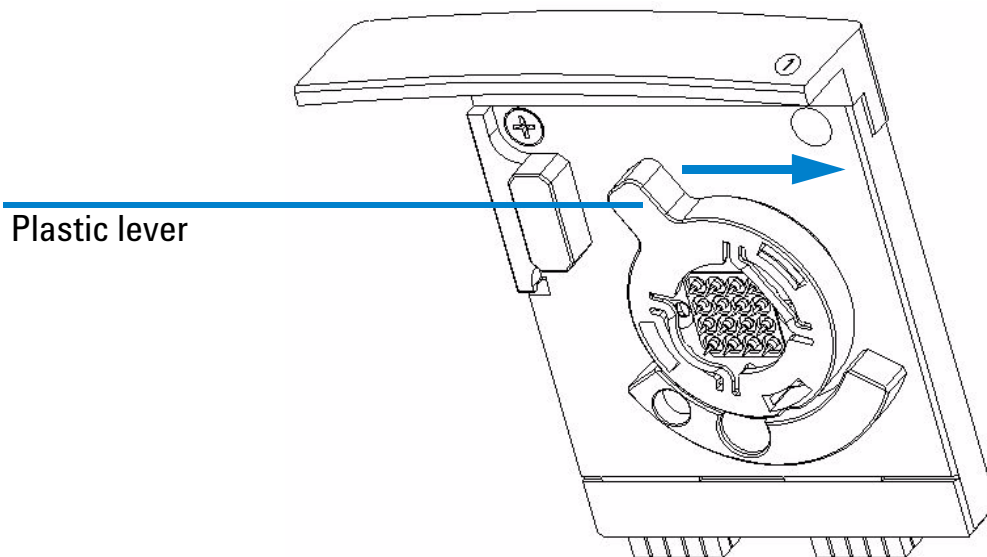
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**10** Put the pin set on the cartridge base and the bayonet cover on the pin set. See Figure 6, “Inserting the Pin Set”.



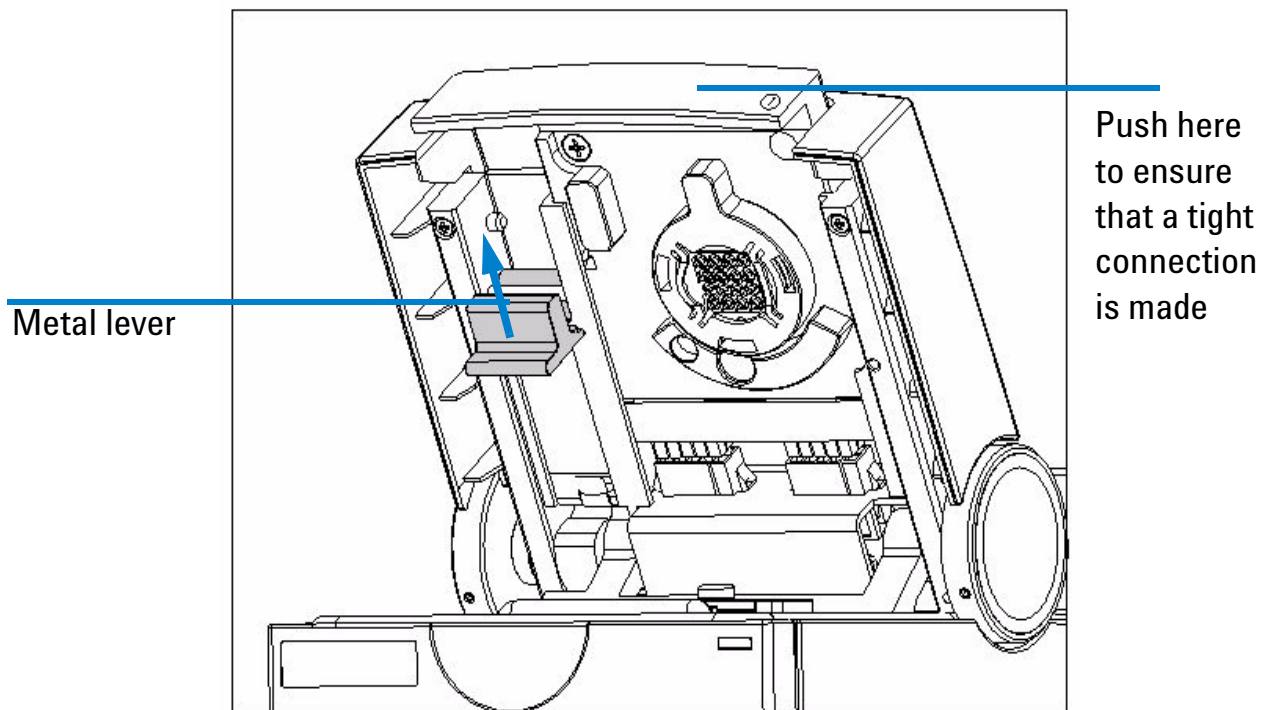
**Figure 6** Inserting the Pin Set

**11** Lock the pin set to the electrode base by pushing the plastic lever of the bayonet cover to the right as shown in Figure 7, “Closing the Socket of the Pin Set”.



**Figure 7 Closing the Socket of the Pin Set**

- 12** Slide the electrode cartridge with the pin-set into the bioanalyzer lid as shown in Figure 8, “Inserting the Electrode Cartridge” and move the metal lever in the flat (closed) position.
- 13** Push the metal front of the electrode cartridge to ensure a tight connection to the 2100 bioanalyzer.



**Figure 8 Inserting the Electrode Cartridge**

- 14 To verify that the electrodes are completely dry, perform the Short Circuit diagnostic test. that you can find in *Diagnostics* tab under the *Instrument* context. This test takes approximately three minutes and the software will walk you through the steps.
- 15 If the short circuit test fails, the assembly may still be wet. Take the assembly out of the instrument, dry it with oil-free compressed air, then repeat the test.

---

### CAUTION

Do not heat the electrode cartridge in an oven. This can permanently damage the electrode cartridge.

---



# Maintenance of the Chip Priming Station

## Overview

Regular cleaning procedures are necessary to maintain the performance of the chip priming station. The following table gives an overview on the different maintenance procedures:

Procedure	Time Interval	or if...
Replacing the Syringe	with each new kit latest every 3 months	...syringe is broken. For details see “ <a href="#">Replacing the Syringe</a> ” on page 203.
Replacing the Syringe Adapter	cleaning: every 3 months	...adapter is clogged with dried gel or damaged. For details see “ <a href="#">Replacing the Syringe Adapter</a> ” on page 204.

Procedure	Time Interval	or if...
Replacing the Gasket	every 3 months	<p>...gasket is damaged, torn or contaminated with dried gel.</p> <p>For details see “<a href="#">Replacing the Gasket</a>” on page 207.</p>
Checking the Chip Priming Station for Proper Performance - Seal Test	every 4 weeks	<p>...gasket, syringe adapter or syringe was replaced.</p> <p>For details see “<a href="#">Checking the Chip Priming Station for Proper Performance - Seal Test</a>” on page 209.</p>

# Replacing the Syringe

**When required** • with each new kit or latest every 3 months

**Material required** • syringe kit that comes with each DNA, RNA and protein kit

## Procedure

- 1 Unscrew the old syringe from the lid of the Chip Priming Station.
- 2 Release the old syringe from the clip. Dispose it according to good laboratory practices.
- 3 Remove the plastic cap of the new syringe and insert it into the clip.
- 4 Slide it into the hole of the luer lock adapter and screw it tightly to the priming station.
- 5 Check the priming station as described in “[Checking the Chip Priming Station for Proper Performance - Seal Test](#)” on page 209.

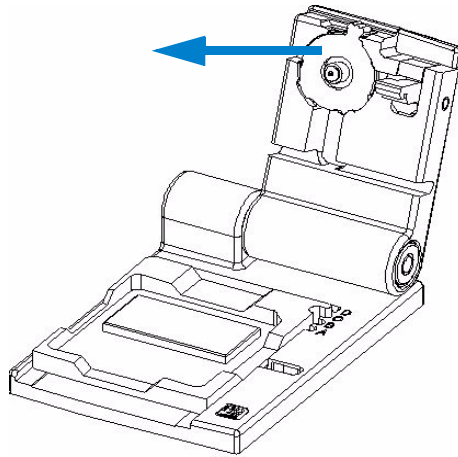
# Replacing the Syringe Adapter

**When required** • every 3 months or whenever it is clogged with dried gel

**Material required** • Gasket kit (reorder no. G2938-68716)

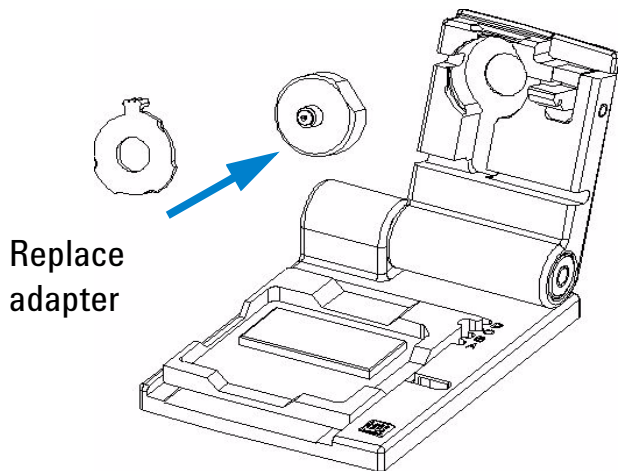
## Procedure

- 1 Remove the syringe by gently pulling it out of the adapter.
- 2 Open the Chip Priming Station.
- 3 Move the ring holding the adapter in place to the left as shown in Figure 9, “Releasing the Mounting Ring of the Syringe Adapter”. The ring will come off.



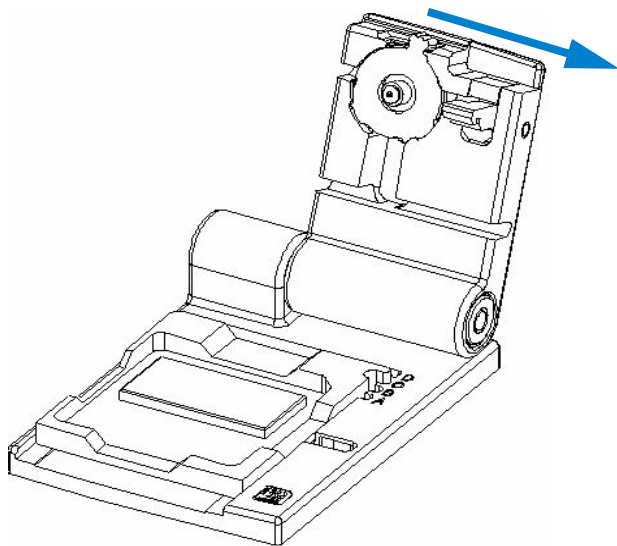
**Figure 9** Releasing the Mounting Ring of the Syringe Adapter

- 4 Press the syringe adapter out of its mount and dispose it according to good laboratory practices, as shown in Figure 10, “Replacing the Syringe Adapter”.
- 5 Insert the new syringe adapter.



**Figure 10 Replacing the Syringe Adapter**

- 6 Insert the mounting ring and move it to the right in order to fix the syringe adapter as shown in Figure 11, “Fixing the Syringe Adapter”.



**Figure 11 Fixing the Syringe Adapter**

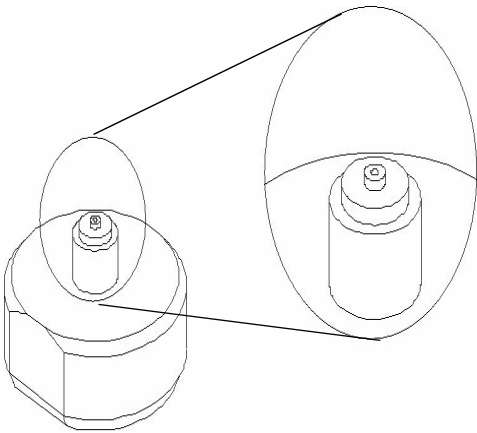
- 7 Close the Chip Priming Station
- 8 Slide the syringe into the hole of the luer lock adapter and screw it tight.
- 9 Check the priming station as described in “[Checking the Chip Priming Station for Proper Performance - Seal Test](#)” on page 209.

# Replacing the Gasket

**When required** • every 3 months or when it is torn or contaminated with dried gel

**Material required** • Gasket kit (reorder no. G2938-68716)

The silicone gasket, see Figure 12, “[Syringe Adapter with Gasket](#)”, ensures a tight connection between chip and syringe adapter.

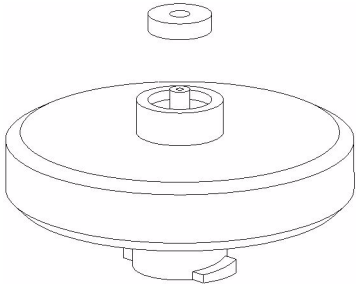


**Figure 12 Syringe Adapter with Gasket**

## Procedure

- 1 Remove the syringe adapter out of the chip priming station as described in “[Replacing the Syringe Adapter](#)” on page 204.

- 2 Pull out the old gasket with your fingers or with the help of a needle. See Figure 13, “[Syringe Adapter with disassembled Silicone Gasket](#)” for a disassembled adapter.



**Figure 13 Syringe Adapter with disassembled Silicone Gasket**

---

**CAUTION**

Avoid scratching the plastic adapter when removing the silicon gasket.

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- 3 Insert new gasket and gently push into place.
- 4 Insert the syringe adapter into the chip priming station as described in “[Replacing the Syringe Adapter](#)” on page 204.
- 5 Check the priming station as described in “[Checking the Chip Priming Station for Proper Performance - Seal Test](#)” on page 209.



# Checking the Chip Priming Station for Proper Performance - Seal Test

**When required**

- every month or whenever a component of the priming station (syringe, adapter or gasket) was replaced

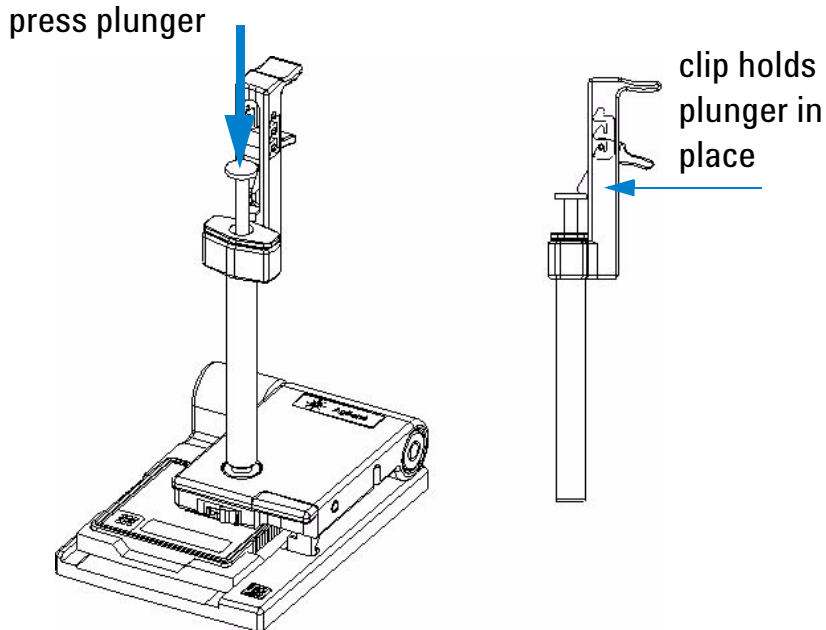
**Material required**

- unused chip

## Procedure

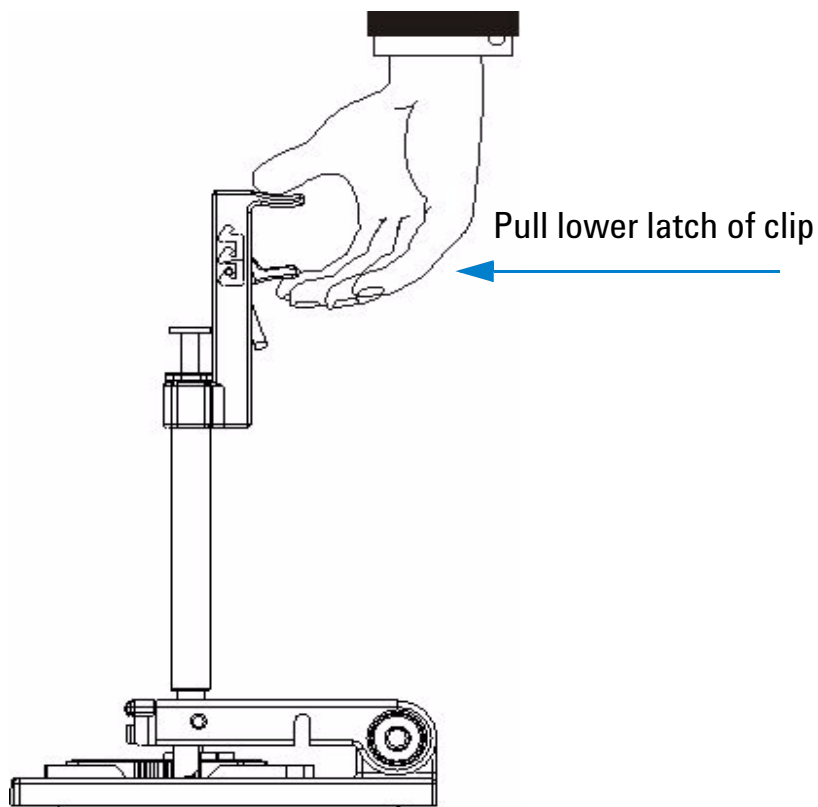
- 1 Make sure the syringe is tightly connected to the chip priming station.
- 2 Pull the plunger of the syringe to the 1.0 ml position (plunger pulled back).
- 3 Place an unused chip in the chip priming station.
- 4 Close the chip priming station and make sure to lock it by pressing the cover. The lock of the latch will audibly click when it closes.

- 5 Press the plunger down until it is locked by the clip. This is shown in Figure 14, “Locking the Plunger of the Syringe with the Clip”.



**Figure 14 Locking the Plunger of the Syringe with the Clip**

- 6 Wait for 5 seconds and press the side of the clip to release the plunger as shown in Figure 15, “Releasing the Plunger of the Syringe”.



**Figure 15 Releasing the Plunger of the Syringe**

- 7** Appropriate sealing is verified, if the plunger moves back up to the 0.3 ml mark within less than 1 second.

---

**NOTE**

If the plunger doesn't move up to the 0.3 ml mark within a second, the syringe-chip connection is probably not tight enough. Re-tighten the syringe or replace the syringe adapter, syringe or gasket to fix the problem.

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# Maintenance of the Pressure Cartridge

## Overview

The following table gives an overview on the different maintenance procedures of the pressure cartridge.

Procedure	Time Interval	or if...
Cleaning the Pressure Adapter	after each run	...gasket is contaminated with dried cell buffer.  For details, see <a href="#">“Cleaning the Pressure Adapter”</a> on page 214.
Replacing the Pressure Adapter	every 12 months	...adapter is clogged and an error message is generated by the software.  For details, see <a href="#">“Replacing the Pressure Adapter”</a> on page 216.

# Cleaning the Pressure Adapter

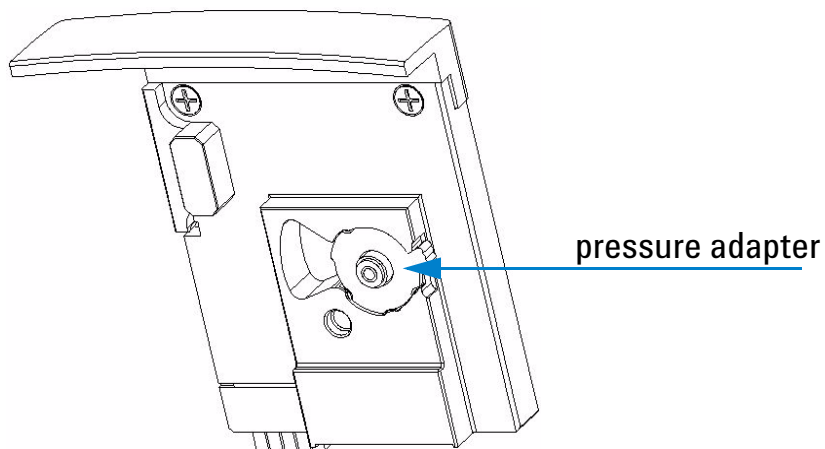
**When required**      • after each cell chip run

**Material required**      • lint-free tissue or cloth

When the chip run is finished, immediately remove the chip out of the Agilent 2100 bioanalyzer. Then perform the following procedure to ensure that the pressure adapter, see Figure 16, "[Pressure cartridge with pressure adapter.](#)" on page 215, is clean and no residues are left over from the previous assay.

## Procedure

- 1 Open the instrument and remove the chip.
- 2 Should there be liquid at the adapter of the pressure cartridge, use a tissue to dry off the gasket. Make sure not to touch the lens.



**Figure 16 Pressure cartridge with pressure adapter.**

---

**NOTE**

Dispose the cell contaminated chip and all other cell contaminated material according to good laboratory practices.

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# Replacing the Pressure Adapter

- When required**
- every 12 months or whenever it is clogged and the software generates an error message
- Material required**
- pressure adapter kit (reorder no 5065-4478)

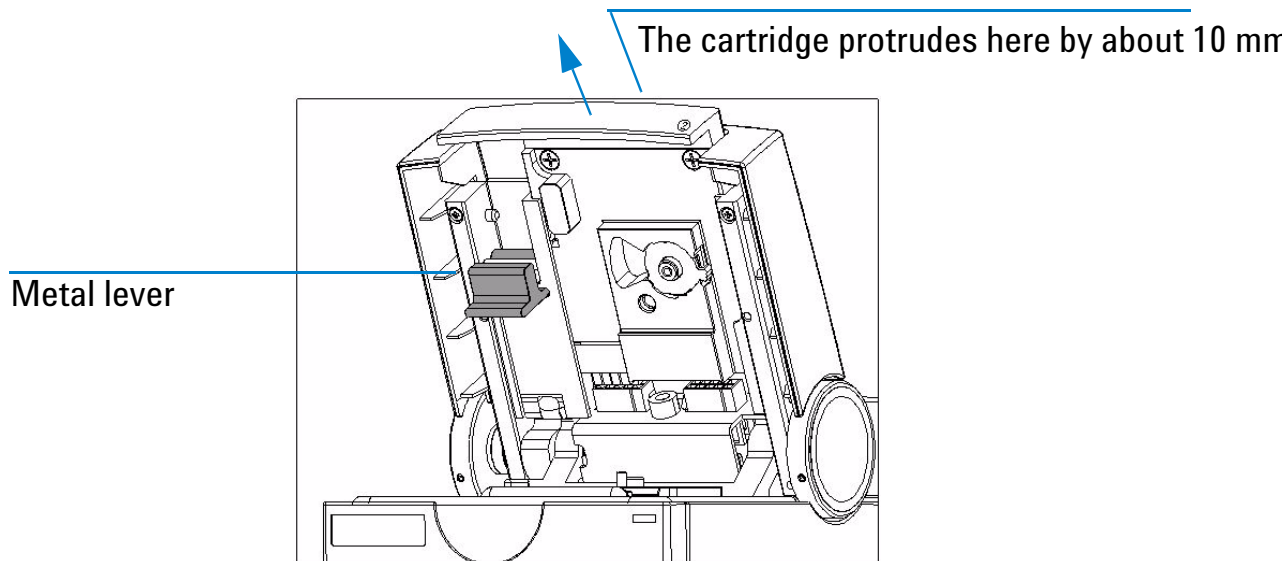
Improper chip preparation might lead to clogging of the pressure adapter which is located in the pressure cartridge.

## Procedure

- 1 Switch off the instrument. The line switch is located at the rear of the 2100 bioanalyzer.
- 2 Open the lid and pull down the locking lever on the left inside the lid to the open position as shown in Figure 17, “[Removing the pressure cartridge](#)”. The cartridge is pushed out about 10 mm.

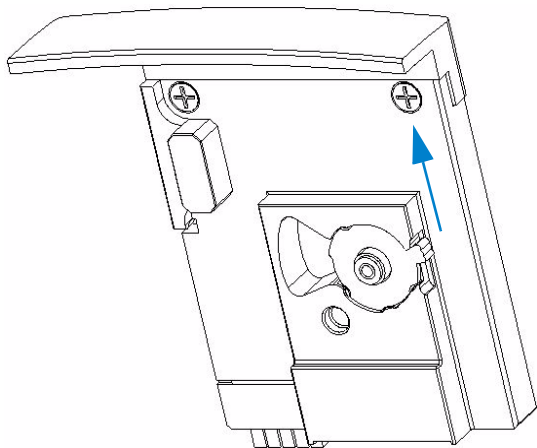


**3** Gently pull the cartridge out of the lid.



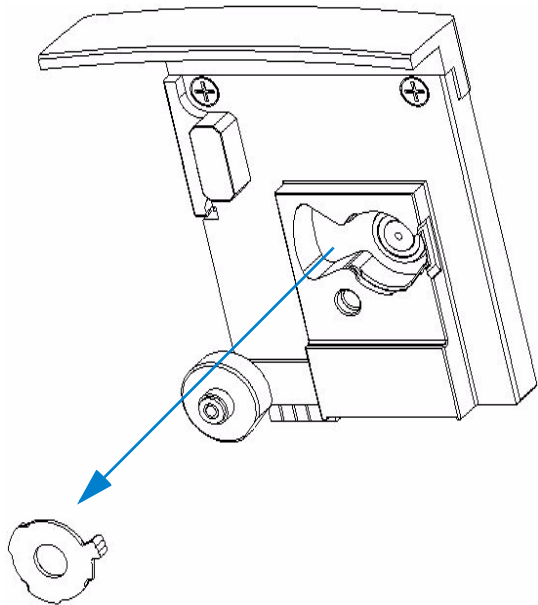
**Figure 17 Removing the pressure cartridge**

- 4 Move the ring holding the adapter in place to the left as shown in Figure 18, “Opening the ring of the pressure cartridge”. The ring will come off.



**Figure 18** Opening the ring of the pressure cartridge

- 5 Pull the pressure adapter out of its mount, and replace it, as shown in Figure 19, “Releasing the pressure adapter”.



**Figure 19 Releasing the pressure adapter**

- 6 Re-insert the new pressure adapter and lock it by the mounting ring. Make sure that the pressure adapter is inserted correctly; it fits only one way.

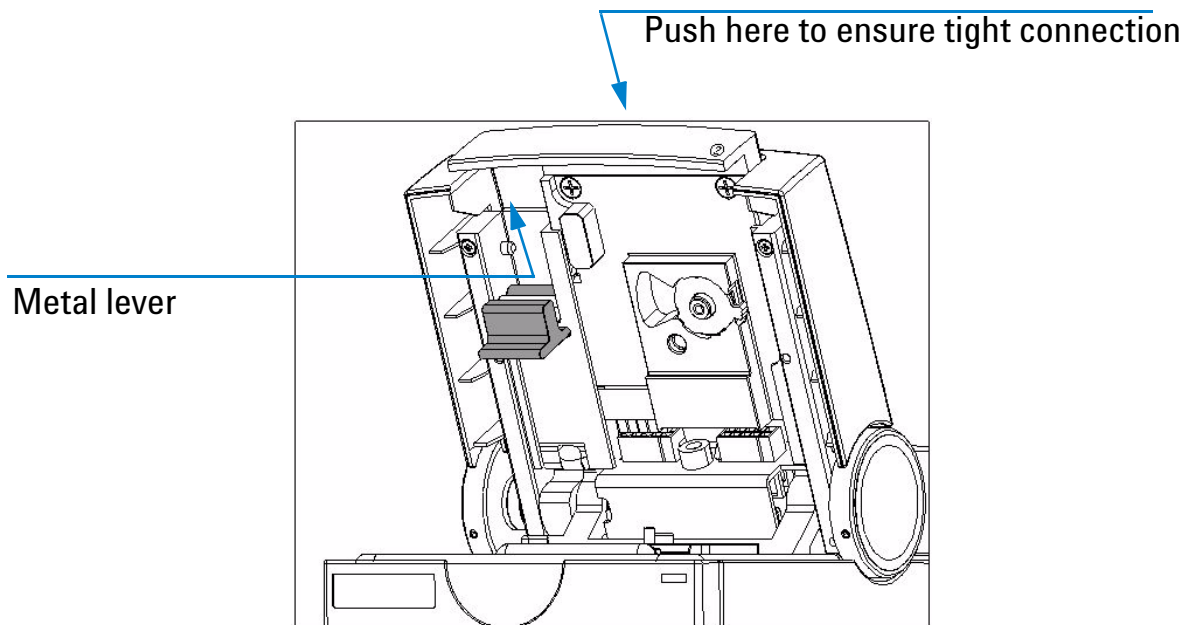
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NOTE

Dispose all cell contaminated material according to good laboratory practices.

---

- 7 Slide the Pressure Cartridge in the lid as shown in Figure 20, “Inserting the pressure cartridge”.
- 8 Move the metal lever in the flat (closed) position.
- 9 Push the metal front of the pressure cartridge to ensure a tight connection to the 2100 bioanalyzer.



**Figure 20 Inserting the pressure cartridge**

# Maintenance of the Agilent 2100 Bioanalyzer

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## WARNING

**If pathogenic, toxic, or radioactive samples are intended to be used in this instrument, it is the responsibility of the user to ensure that all necessary safety regulations, guidelines, precautions and practices are adhered to accordingly. Ask your laboratory safety officer to advise you about the level of containment required for your application and about proper decontamination or sterilization procedures to follow if fluids escape from containers. For further safety information please refer to the 2100 Bioanalyzer Site and Safety Manual.**

---

The Agilent 2100 bioanalyzer should be kept clean. Cleaning should be done with a damp lint-free cloth. Do not use an excessively damp cloth allowing liquid to drip into the Agilent 2100 bioanalyzer. The following table gives an overview on the different 2100 bioanalyzer maintenance procedures:

Procedure	Time Interval	of if...
Cleaning the Lens	latest every 3 months	...lens is contaminated with liquid spill
Changing the Fuses of the Agilent 2100 Bioanalyzer	n/a	...status indicator is off and the cooling fan is not running

# Cleaning the Lens

**When required** • every 3 months or after liquid has been spilled on the lens

**Material required** • reagent-grade isopropanol

- lens tissue

Liquid spill may reduce the light throughput of the focusing lens underneath the chip. To avoid low intensity signals because of absorbing coatings on the lens, follow the procedure below.

## Procedure

- 1 Switch off the instrument. The line switch is located at the rear of the 2100 bioanalyzer.
- 2 Open the lid of the instrument.
- 3 Dampen a lens tissue with reagent-grade isopropanol and gently swab the surface of the lens. Repeat several times with clean tissues and alcohol each time.

---

### CAUTION

Do not let liquid drip into the Agilent 2100 bioanalyzer. It could cause a shock or it could damage the instrument.

---

- 4 Wait for alcohol to evaporate before use.

# Changing the Fuses of the Agilent 2100 Bioanalyzer

**When required** • if the status indicator is off and the cooling fan is not running

**Material required** • 2 fuses 1A 250V (reorder no. 2110-0007)

**Tools required** • screwdriver

## Procedure

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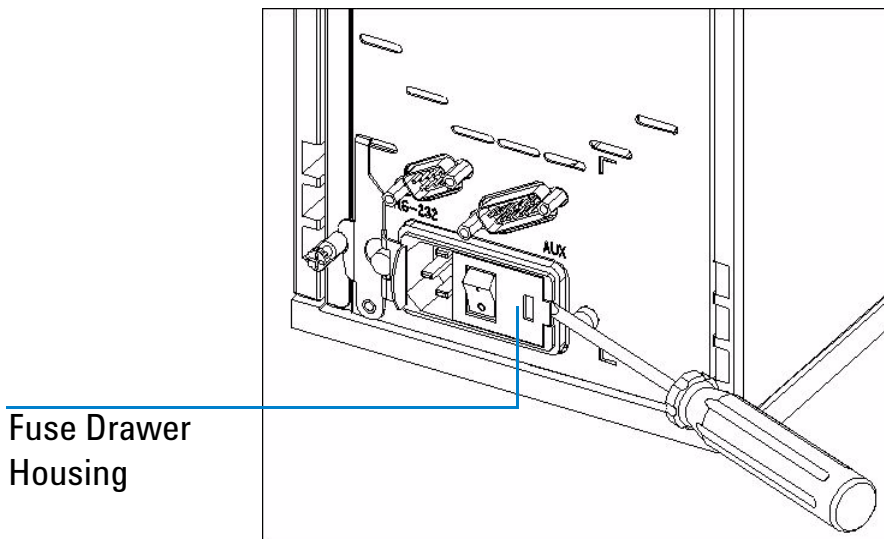
### CAUTION

Disconnect the Agilent 2100 bioanalyzer from line power before changing a fuse.

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- 1 Switch off the instrument. The line switch is located at the rear of the 2100 bioanalyzer.
- 2 Disconnect the power cable from the power input socket.

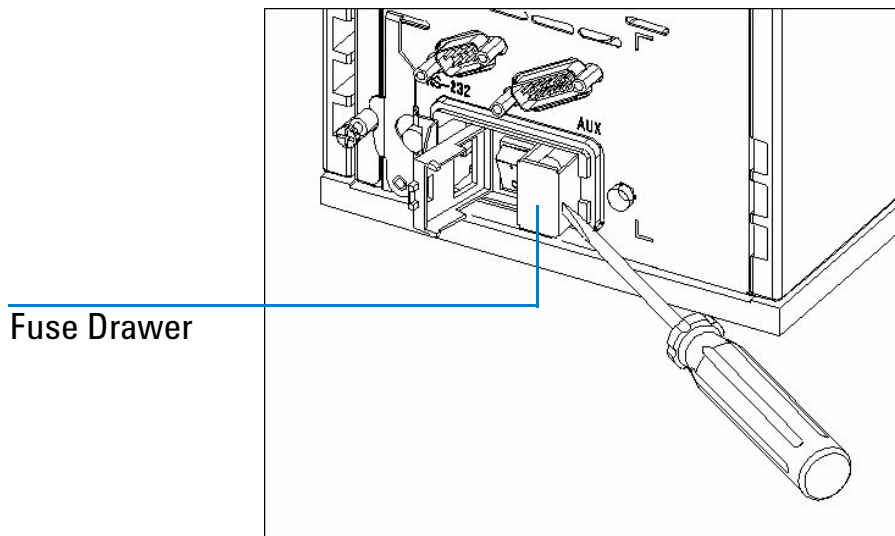
- 3 To access the fuse drawer, gently lift the outer plastic housing of the power inlet socket using a screwdriver, see Figure 21, “Lifting the Housing of the Power Inlet”.



**Figure 21 Lifting the Housing of the Power Inlet**



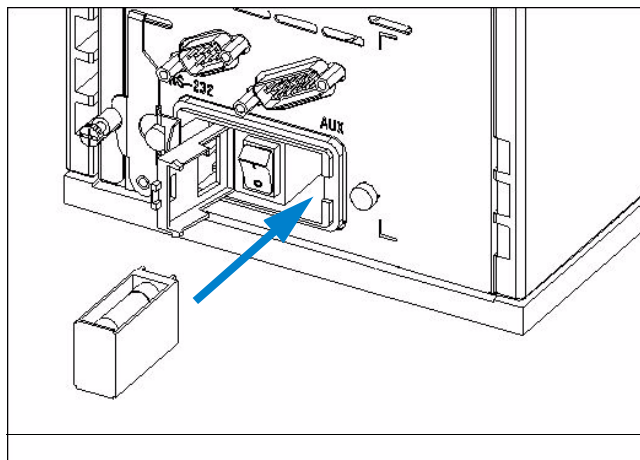
4 Pull out the fuse drawer as shown in Figure 22, “Pulling out the Fuse Drawer”.



**Figure 22 Pulling out the Fuse Drawer**

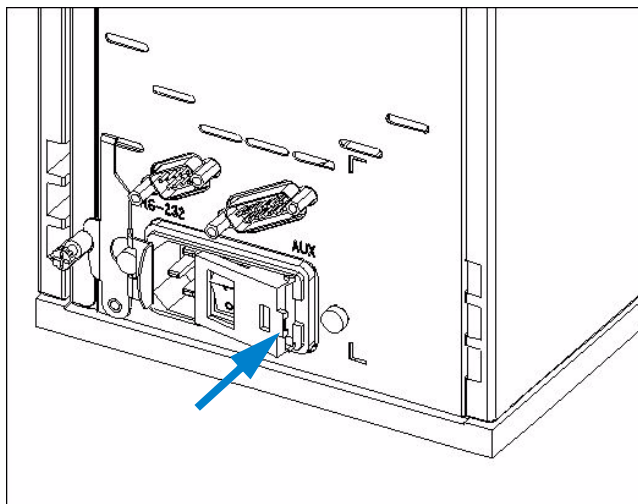
5 Replace the 2 fuses.

- 6 Slide in the fuse drawer and push till it fits tightly as shown in the Figure 23, “Slide in the Fuse Drawer”.



**Figure 23 Slide in the Fuse Drawer**

- 7 Finally, close the fuse drawer housing, reconnect the instrument to the power line and switch it on.



**Figure 24 Close Fuse Drawer Housing**

# Maintenance of the Vortexer: Changing the Adapter

- When required**
- whenever the adapter is damaged
- Material required**
- IKA Vortex Mixer Adapter (reorder-no. 5022-2190)
- Tools required**
- screwdriver

## Procedure

- 1 Release the 3 screws on top of the adapter
- 2 Hold the base of the vortex mixer and pull up the head. Discard the old head according to good laboratory practices.
- 3 Place the new head adapter on the vortex mixer.
- 4 Insert and fix the 3 screws with the screwdriver.

[Print end:](#)

# Glossary

The glossary explains terms used in context with application, software, or hardware of the Agilent 2100 bioanalyzer.

## **XAD Files**

Raw data is stored as .xad files. The files contain raw data, assay informations and the run log information.

## **Serial Port**

The serial ports are used to connect your computer with the Agilent 2100 bioanalyzer. The number of available ports depends on the system you use.

## **CSY Files**

Assays are stored as .csy files. The files contain the assay properties, data acquisition settings and the sample names.

## **Firmware**

The firmware is a program to control the hardware of the Agilent 2100 bioanalyzer. It is downloaded from your computer to the Agilent 2100 bioanalyzer and controls the data transfer or the measurement procedures, for example.

## **Flow Cytometry**

A method to detect cells with certain properties. In a continuous stream, stained cells pass through a light beam. The emitted fluorescence is used for counting and differentiation.

### **Flow Cytometry Standard (FCS)**

The FCS file format is the standard format used in flow cytometry to exchange data between several applications.

### **Histogram**

Histograms are bar charts to display a frequency distribution, for example.

### **PCK Files**

Packet files (.pck) contain all data transferred during measurement. The files are stored in the packet folder of the installation directory. Together with the log files they can be used to restore lost or destroyed data by the Agilent service.

# Spare Parts and Accessories

The following table provides a list of spare parts and accessories that are available for the Agilent 2100 bioanalyzer. To buy parts, please refer to the Agilent Online Store:

<http://www.agilent.com/home/buyonline.html>

Reorder number	Part	Description
G2938-90300	Kit Guide Binder	contains all available reagent kit guides in a transparent plastic binder
G2941-60027	Bioanalyzer How to Use CD-ROM	contains videos showing assay preparation and maintenance of bioanalyzer and accessories
5065-9951	Electrode Cleaner Box	contains 7 Electrode Cleaners for the maintenance of the electrode cartridge
G2938-81605	RS 232 Cable	communication cable between PC and instrument
G2938-81610	Multiport Cable	for rocketport card
2110-0007	Fuse	fuse for power supply
5065-4401	Chip Priming Station	includes gasket kit and adjustable clip
G2938-68716	Gasket Kit	contains spare parts for chip priming station: 1 plastic adapter, 1 ring and 10 gaskets

<b>Reorder number</b>	<b>Part</b>	<b>Description</b>
5042-1398	Adjustable Clip	for use with luer lock syringe
5065-4413	Electrode Cartridge	no extra electrode pin set; pin-set not re-orderable
5065-4492	Pressure Cartridge	for use with flow cytometry assays
5065-4478	Pressure Adapter Kit	contains 5 pressure adapters for use with the pressure cartridge
G2938-68100	Test Chip Kit for Molecular Assays	comprises 1 Autofocus, 1 Electrode/Diode, 5 Leak Current Clips
G2938-68200	Test Chip Kit for Cell Assays	comprises 1 Cell Autofocus Chip
5022-2190	Vortex Mixer Adapter	for IKA vortexer
5065-4428	IKA Vortexer (115V)	must be ordered at IKA
5065-4429	IKA Vortexer (230V)	must be ordered at IKA



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