Agilent Cell Checkout Kit

Kit Guide

Agilent Technologies

Notices

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Software Revision

This guide is valid for B.01.02 and B.02.02 and higher revisions of the Agilent Expert software, where 02 refers to minor revisions of the software that do not affect the technical accuracy of this guide.

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CAUTION

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WARNING

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.

Contents

Agilent Cell Checkout Kit

Contents of the Agilent Cell Checkout Kit:

Agilent Cell Checkout Kit (reorder number 5067-1520)
Cell Assay Checkout Chips
7 Chips
Cell Assay Checkout Reagents
● Cell Buffer
● Focusing Dye Solution
❍ Chip Priming Solution
● Blue Beads

Cell Checkout Kit: Specifications	
Analysis run time	30 minutes
Number of samples	6
Sample volume	10 µl
Assay kit stability	4 months at 4 °C

Equipment Required for a Cell Checkout Assay

Equipment supplied with the Agilent 2100 bioanalyzer

- Agilent 2100 Bioanalyzer with SN above DE 137001001 (G2938B or G2938C)
- Cell Assay Extension (G2944AA) or Flow Cytometry Set (G2948CA)
- Vortex mixer

Additional material required (not supplied)

- Pipettes (10 µl, 100 µl and 1000 µl) with compatible tips
- 1.5 ml or 0.5 ml microcentrifuge tubes for bead preparation

Setting up the Bioanalyzer

Before beginning the chip preparation protocol, ensure that the bioanalyzer is set up and ready to use.

You have to

- verify that the bioanalyzer has the pressure cartridge inserted
- enter the flow cytometry licence key
- adjust the bioanalyzer's chip selector and
- start the 2100 expert software.

NOTE The Agilent Cell Assay is a high sensitivity assay. Please read this guide carefully and follow all instructions to guarantee satisfactory results.

Setting up the Bioanalyzer

NOTE Use cell chips only with Agilent 2100 bioanalyzer with SN above DE137001001 and a pressure cartridge. Only Agilent 2100 bioanalyzer models G2938B or G2938C support flow cytometric applications.

Adjust the chip selector:

1. Open the lid of the bioanalyzer and make sure that the pressure cartridge is inserted in the instrument.
2. Remove any remaining chip and adjust the chip selector to position (2).

NOTE Do not use the chip selector in position 1. This position refers to electrophoretic assays (DNA, RNA and protein assays).

Starting the 2100 Expert Software

NOTE Login is required when SP is activated. Flow cytometry licence and the instrument control licence are required to enter the 2100 Expert software. Beside that the electrophoresis licence can be there as well to allow easy switch between electropheresis and flow cytometric assays.

To start the software:

1 Go to your desktop and double-click the following icon.

The screen of the software appears in the *Instrument context*. The icon in the upper part of the screen represents the current instrument-PC communication status:

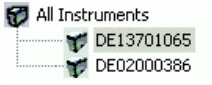

Lid closed, no chip or chip empty


Lid open


Dimmed icon: no communication

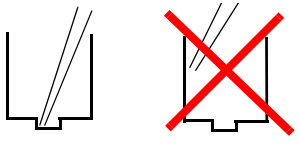

Lid closed, chip inserted, cell fluorescence or demo assay selected

2 If more than one instrument is connected to your PC, select the instrument you want to use in the tree view of the *instrument context*.



Essential Measurement Practices

- Handle and store all reagents according to the instructions on the label of the individual box.
- Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.
- Keep all reagent and reagent mixes refrigerated at 4 °C when not in use.
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes before use.
- Protect beads and focusing dye from light. Remove light covers only when pipetting. The dye decomposes when exposed to light and this reduces the signal intensity.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well may lead to poor results.



- For chip preparation, use inverse pipetting.

NOTE

Inverse pipetting:
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. Do not touch the Agilent 2100 bioanalyzer during analysis and never place it on a vibrating surface.

- Never leave any wells empty, or the pressure adapter may become clogged. Pipette 10 µl of cell buffer or sample replicate in any empty sample well.
- Before bead preparation, vortex bead vials for 15 s.
- Prepared chips must be used within 5 minutes. If a chip is not run within 5 minutes reagents may evaporate leading to poor results.
- Don't touch the Agilent 2100 bioanalyzer during a run and never place it on a vibrating surface.
- Never touch the instrument lens. Refer to the *2100 Expert Maintenance & Troubleshooting Guide* for lens maintenance.

Agilent Checkout Assay Protocol

After completing the initial steps in "Setting up the Bioanalyzer" on page 7, you can prepare the assay, load the chip, and run the assay, as described in the following procedures.

NOTE

For hints on staining optimization, handling or experimental setup check for detailed newest Application Notes at www.agilent.com/chem/labonachip
or within the *2100 Expert help menu* in the list of related documents.

WARNING

Handling reagents

The handling of reagents and chemicals might hold health risks.

⇒ Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.

⇒ All reagents should be handled with care ususal when dealing with chemicals.

For further chemical and biological safety information please refer to the Agilent Technologies 2100 Bioanalyzer Installation and Safety Manual.

Preparing the Beads

1 Allow all reagents to equilibrate to room temperature for 30 minutes before use.

2 Vortex bead vial for 15 seconds.

3 Pipette 95 µl of cell buffer (● green) into a 0.5 ml microcentrifuge tube.

4 Add 5 µl of blue beads (● blue).

5 Vortex for 15 seconds.

NOTE Store the beads in the dark at room temperature and use within one day.

6 Load the prepared beads onto the chip after loading the chip priming solution, focusing dye solution and cell buffer.

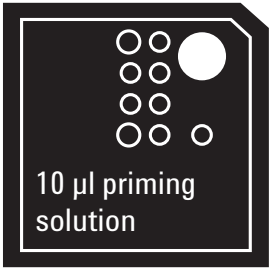
Loading the Chip Priming Solution

NOTE No priming station is required to prime the cell chip but you may use it to hold the chip in place during loading.

1 Take a new chip out of its sealed bag and place the chip on the Chip Priming Station.

2 Pipette 10 µl of the chip priming solution (❍ white) into the large priming well (PS). Insert the tip of the pipette to the bottom of the well when dispensing. Use inverse pipetting.

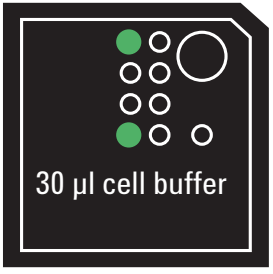
3 Wait for 60 seconds. Capillary force fills all channels of the chip.



Loading the Focusing Dye Solution, Cell Buffer and Beads

1 Pipette 10 µl of focusing dye solution (● yellow) into the focusing well (FD). Insert the pipette tip to the bottom of the well when dispensing. Use inverse pipetting.

2 Pipette 30 µl of cell buffer (● green) into each of the 2 cell buffer wells (CB). Use inverse pipetting procedure to avoid introduction of air bubbles.



NOTE By pipetting 30 µl of cell buffer, the cell buffer wells will be quite full.

3 Pipette 10 µl of beads (prepared as described in chapter on "Preparing the Beads" on page 12 into each of the 6 sample wells (1-6).
Use inverse pipetting procedure.

NOTE Do not leave any wells empty or the chip will not run properly. If less than 6 samples are to be used, place 10 µl of cell buffer into the empty sample wells.

NOTE Make sure that the run is started within 5 minutes.

Inserting a Chip in the Agilent 2100 Bioanalyzer

1 Open the lid of the Agilent 2100 bioanalyzer.

2 Check that the pressure cartridge is inserted properly and the chip selector is in position (1). Refer to "Setting up the Bioanalyzer" on page 7 for details.

3 Place the prepared chip carefully into the receptacle. The chip fits only one way.

4 Carefully close the lid.

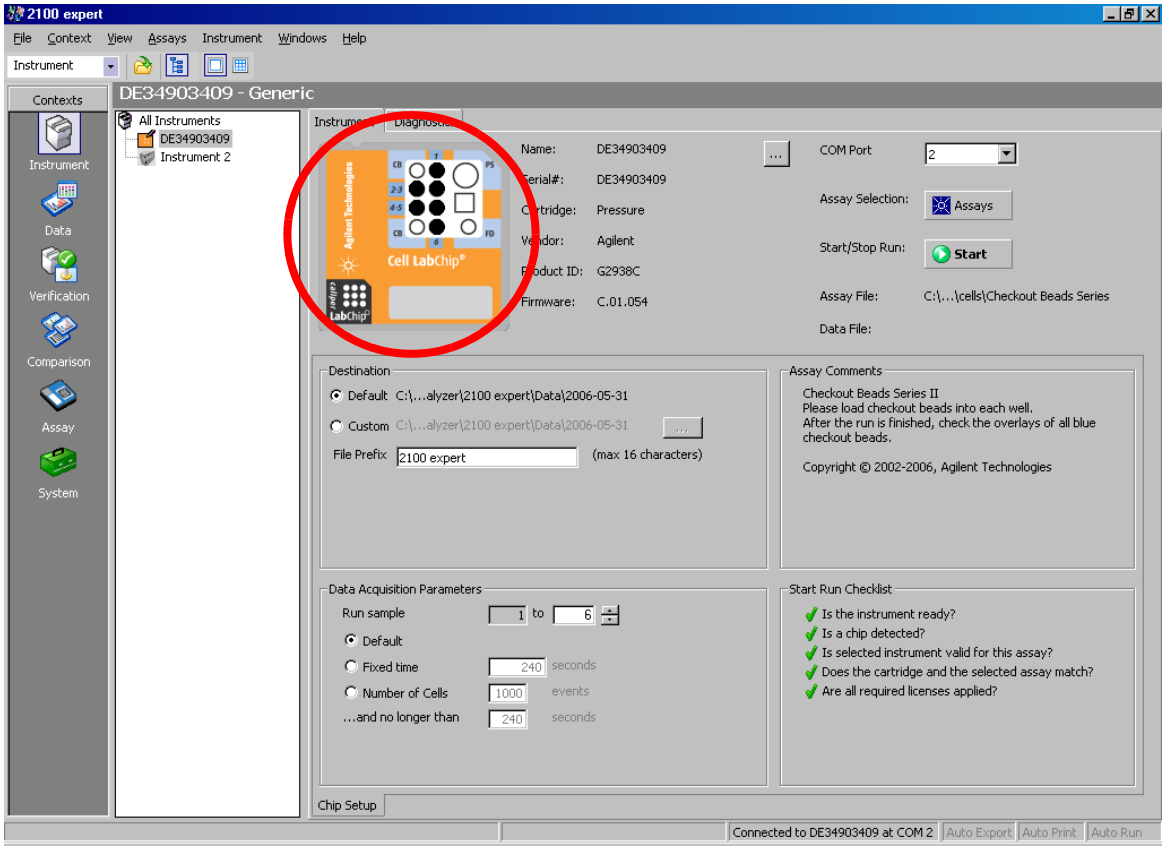
NOTE There may be a small gap between lid and instrument. This does not disturb the measurement as long as the chip is sealed completely.

CAUTION *Sensitive adapter/cartridge*

Forced closing of the lid may damage the adapter or cartridge.

⇒ Do not use force to close the lid.

5 The 2100 expert software screen shows that you have inserted a chip and closed the lid by displaying the chip icon at the top left of *Instrument* context.

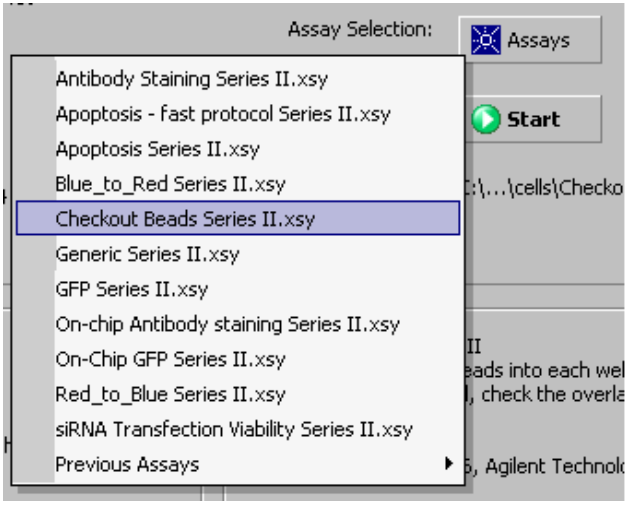


NOTE If the chip is not detected, open and close the lid again.

Starting the Chip Run

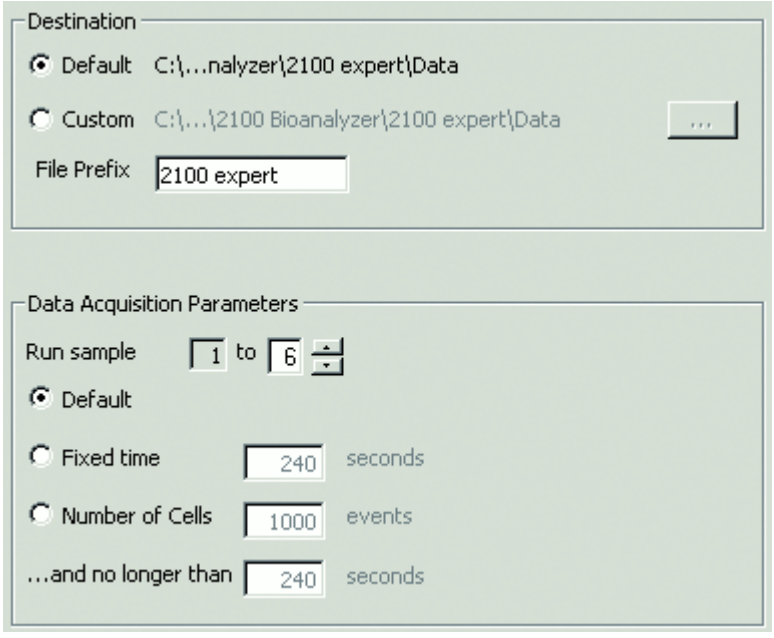
NOTE Please note that the order of executing the Chip Run may change if the Agilent Security Pack software (only applicable for Agilent 2100 Expert software Revision B.02.02 and higher) is installed. For more details please read the 'User's Guide' which is part of the Online Help of your 2100 Expert Software.

1 In the *Instrument* context, select the appropriate assay from the Assay menu.



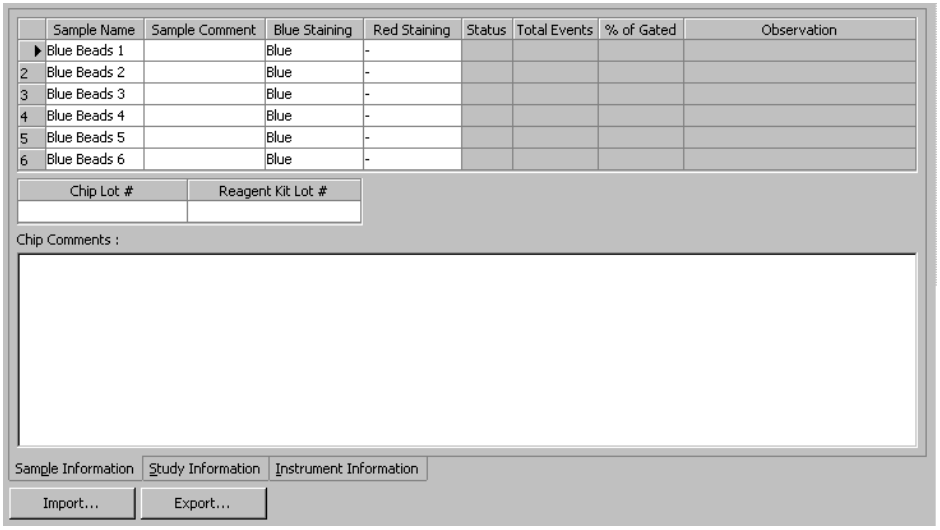
2 Accept the current *File Prefix* or modify it.

Data will be saved automatically to a file with a name using the prefix you have just entered. At this time you can also customize the file storage location and the number of samples that will be analyzed. For changing the data acquisition settings refer to the ***Online Help*** or *2100 Expert User's Guide*.



3 Click the *Start* button in the upper right of the window to start the chip run. The incoming raw signals are displayed in the *Instrument* context.

4 To enter sample information like sample names and comments, select the *Data File* link that is highlighted in blue or go to the *Data and Assay* context and select the *Chip Summary* tab. Complete the sample name table and press *Apply*.



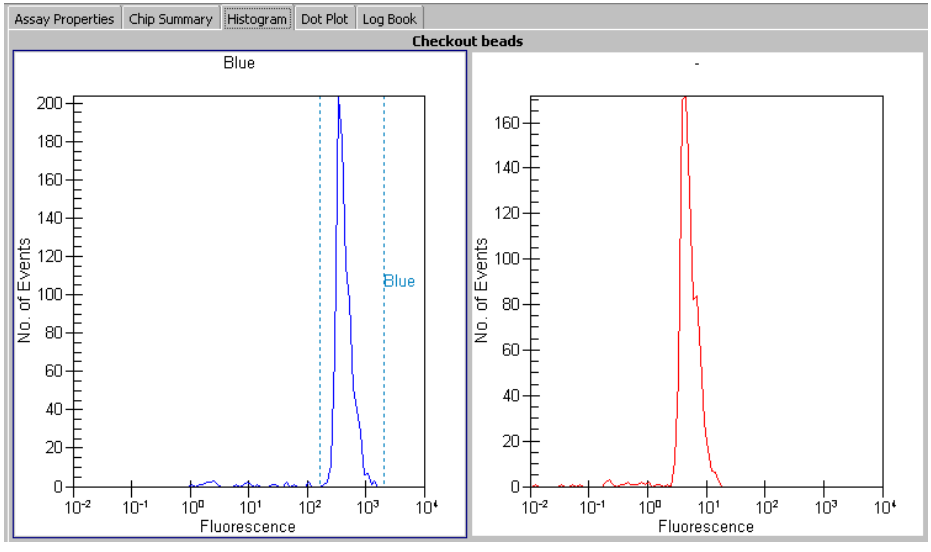
5 To review the raw signal trace, return to the *Instrument* context.

6 After the chip run is finished (*End of Run* message appears.) remove the chip from the receptacle of the bioanalyzer and dispose it according to good laboratory practices.

7 Should there be liquid on the silicone gasket of the cartridge, use a tissue to dry off the gasket. Make sure not to touch the lens.

Checking Your Agilent Checkout Assay Results

To check the results of your run, select the *Data and Assay* context. Data is displayed in histogram or dot plot view. To review the results of a specific sample well, select the sample name in the tree view and highlight the *Histogram* or *Dot Plot* tab. Note that the blue beads will also show a signal in the in the red fluorescence channel.



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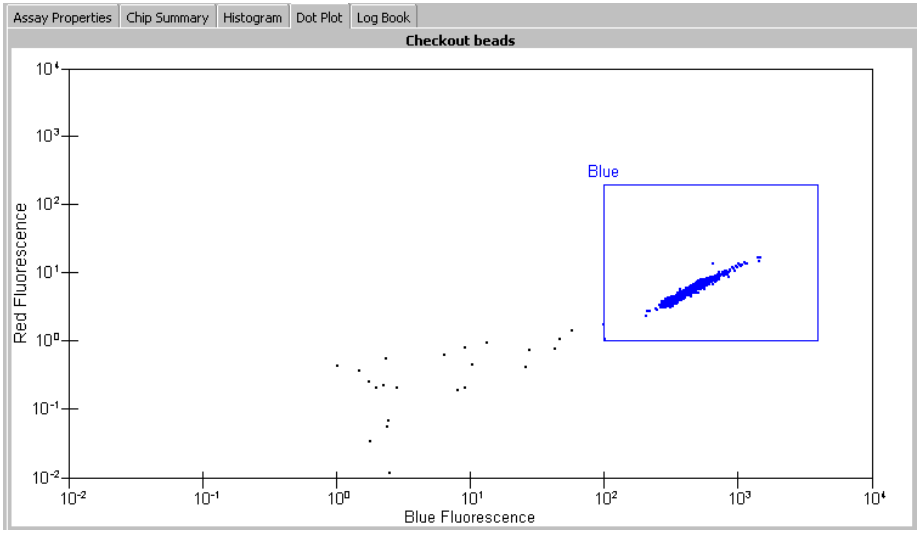

For more details on data evaluation, see the *2100 Expert User's Guide* or *Online Help*.

NOTE For troubleshooting the Cell Application visit the Agilent 2100 Bioanalyzer Maintenance and Troubleshooting section within the 2100 Expert help menu.

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In This Book

you find the procedures to analyze cell samples with the Agilent cell checkout kit and the Agilent 2100 expert bioanalyzer.

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