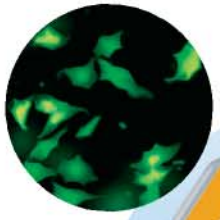


Agilent Cell Assay Kit Guide

Agilent Technologies

Notices

Manual Part Number

G2938-90072

Edition

06/06

Printed in Germany

Agilent Technologies
Hewlett-Packard-Strasse 8
76337 Waldbronn

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.

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

If software is for use in the performance of a U.S. Government prime contract or subcontract, Software is delivered and licensed as "Commercial computer software" as defined in DFAR 252.227-7014 (June 1995), or as a "commercial item" as defined in FAR 2.101(a) or as "Restricted computer software" as defined in FAR 52.227-19 (June 1987) or any equivalent agency regulation or contract clause. Use, duplication or disclosure of Software is subject to Agilent Technologies' standard commercial license terms, and non-DOD Departments and Agencies of the U.S. Government will receive no greater than Restricted Rights as defined in FAR 52.227-19(c)(1-2) (June 1987). U.S. Government users will receive no greater than Limited Rights as defined in FAR 52.227-14 (June 1987) or DFAR 252.227-7015 (b)(2) (November 1995), as applicable in any technical data.

Safety Notices

CAUTION

A **CAUTION** 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 damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

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 Assay Kit

Contents of the Agilent Cell Fluorescence kit:

Agilent Cell Assay Kit (reorder number 5067-1519)
Cell Assay Chips
25 Chips
Cell Assay Reagents
❍ Priming Solution
● Focusing Dye
● Cell Buffer (2 bottles)
● 2x Cell Buffer (2 vials)

NOTE

The 2x cell buffer (● purple) is supplied for measurement with very few cells. The procedures are described in application notes only.

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

The Cell Fluorescence LabChip® Kit together with the Cell Assay Extension or Flow Cytometry Set enables the analysis fluoresecently stained cells.

For application specific protocols and recommended staining reagents please refer to available application notes (http://www.agilent.com/chem/labonachip).

Equipment Required for a Cell Assay

Equipment supplied with the Agilent 2100 bioanalyzer

- Agilent 2100 Bioanalyzer with SN above DE 13701001 (G2938B or G2938C)
- Cell Assay Extension (G2944AA) or Flow Cytometry Set (G2948CA)
- IKA Vortex Mixer

Additional material required (not supplied)

- For cell preparations: 15 ml Falcon tubes, 1.5 ml or 0.5 ml microcentrifuge tubes
- Pipettes (10 µl, 100 µl and 1000 µl) with compatible tips
- Compatible Centrifuge
- Cell counting chamber
- Application (e.g. apoptosis) specific reagents
- Cell strainers

Check the Agilent Lab-on-a-Chip webpage for details on assays:
www.agilent.com/chem/labonachip.

Setting up the Assay Equipment and Bioanalyzer

Before starting the Agilent cell assay, ensure that the Agilent 2100 bioanalyzer is set up and ready to use.

You have to

- verify that the bioanalyzer has the pressure cartridge inserted
- adjust the bioanalyzer's chip selector and
- verify that the flow cytometry licence has been entered.
- 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 electrophoresis 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

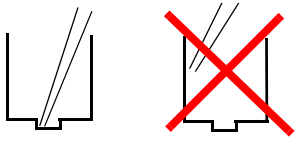

Dimmed icon: no communication


Lid closed, and 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 dye and and dye mixtures 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 cartridge may become clogged. Pipette 10 µl of cell buffer or sample replicate in any empty sample well.
- For optimal results, samples should not exceed cell concentrations of 2.0 million cells per ml.
- On-chip staining requires 3.0 million cells/ml.
- Prepared chips must be used within 5 minutes unless on-chip staining protocol is followed.
- Do not 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.
- Handle cells with minimum shear forces, e. g. choose pipet tips with wide holes.

Agilent Cell Assay Protocol

After completing the initial steps in "Setting up the Assay Equipment and Bioanalyzer" on page 6, 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 appropriate care usual 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 Cells

NOTE The cell sample must be stained with fluorescence dye before beginning with this procedure. For information on the on-chip staining procedure please refer to "Protocol Modifications for On-Chip Staining" on page 20.

1. Treat the cell samples and pellet them according to the application specific protocol.
2. Carefully remove the supernatant without disturbing the pellet.
3. Add an appropriate volume of green-labeled cell buffer (● green) to dilute to a final concentration of 2.0 million cells per ml. On-chip staining requires 3.0 million cells/ml.
4. Resuspend the cell pellet. Depending on the cell type, resuspend the cells either by gentle vortexing or pipetting.
   Check visually if there are any cell clumps or agglomerates left. If so, repeat this step. Make sure that all cell samples are well resuspended.

NOTE If cell clumps or agglomerates cannot be removed (i.e. by vortexing), use cell strainers (40 µm) to filter the cell suspension before loading on the chip.

5. Load the cells onto the chip after loading the chip priming solution, focusing dye solution and cell buffer.

NOTE The cell buffer can not be used to wash cell preparation because cell cannot be pelleted in cell buffer using a centrifuge.

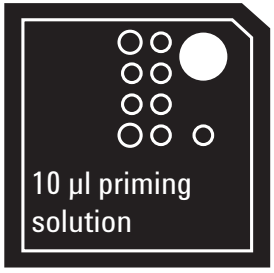
Refer to the dedicated application notes if you want to use on-chip staining procedure with 2x cell buffer.

Loading the Chip Priming Solution

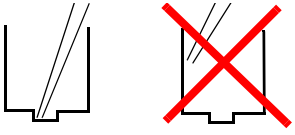
NOTE No priming station is required to prime the cell chip but it may be used to hold the chip in place during loading..

1 Take a new chip out of its sealed bag.

2 Pipette 10 µl of the chip priming solution (❍ white) into the center of the large priming well (PS).



NOTE To prevent the formation aof air bubbles insert the tip of the pipette to the bottom of the chip well when dispensing. Placing the pipette at the edge of the well may lead to poor results.

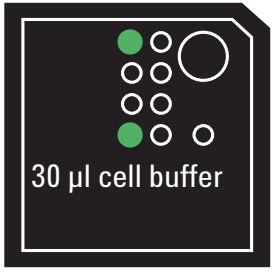


3 Wait for 60 seconds. Capillary force fills all channels of the chip.

Loading the Focusing Dye Solution, Cell Buffer and Samples

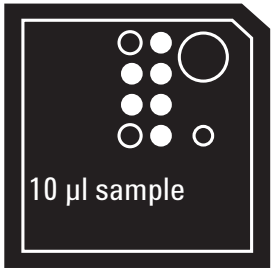
1 Pipette 10 µl of focusing dye solution (● yellow) into the focusing well (FD). Insert the pipette tip to the bottom of the well when dispensing and use inverse pipetting

2 Pipette 30 µl of cell buffer (● green) into each of the 2 cell buffer wells (CB).



NOTE When pipetting 30 µl of cell buffer, the cell buffer wells will be quite full. Do not use the 2x cell buffer here. 2x CB (● purple) concentration is too high. It is supplied with the kit for enabling measurements with few cells. Procedures are described in dedicated application notes.

3 Pipette 10 µl of sample into each of the 6 sample wells (1-6). Insert the tip of the pipette to the bottom of the well when dispensing and use inverse pipetting



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 or a sample replicate 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 (2). 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.

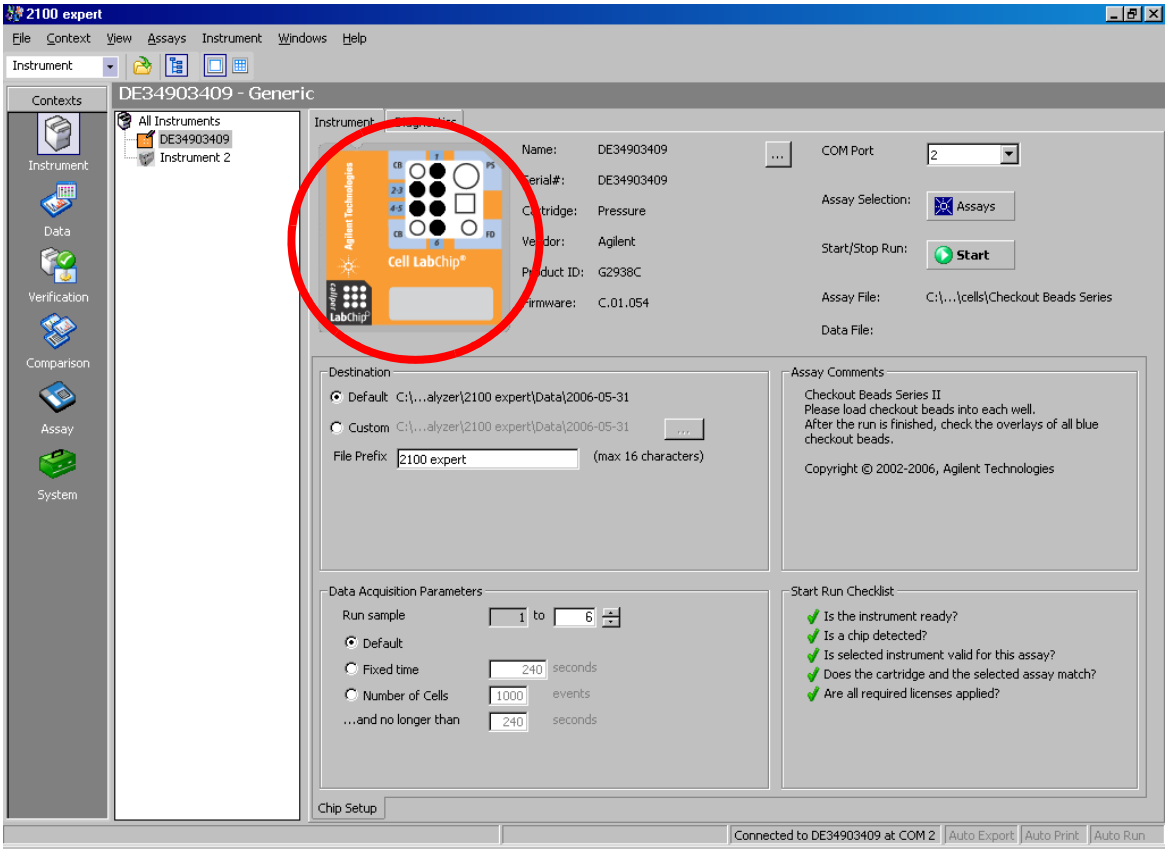
NOTE There may be a small gap between lid and instrument. This does not affect the measurement as long as there is a tight seal between chip and cartridge.

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.

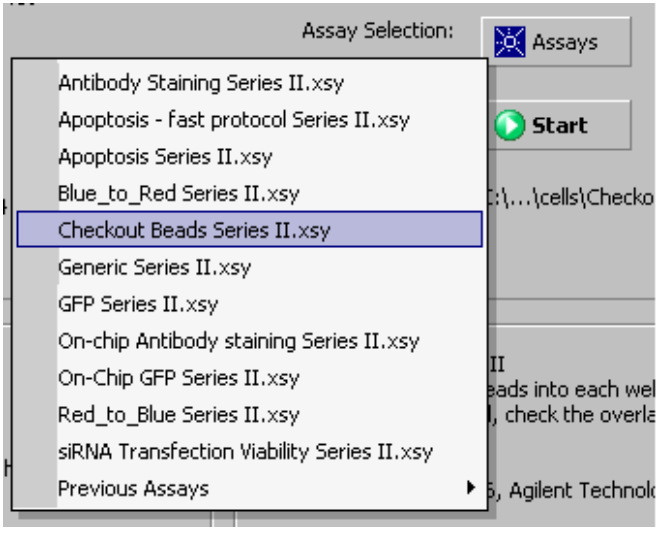


NOTE If the chip is not detected, open and carefully close the lid again.

Starting the Chip Run

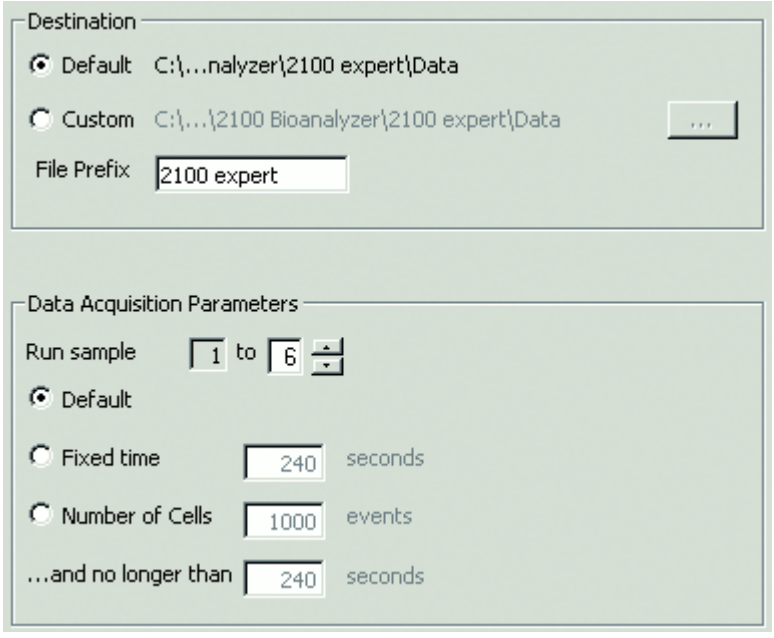
NOTE Please note that the order of executing the Chip Run may change if the Agilent Security Pack software (only applicable for Agilent 2100 Expert software Revision B.02.02 and higher) is installed. For more details please read the 'User's Guide' which is part of the Online Help of your 2100 Expert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

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



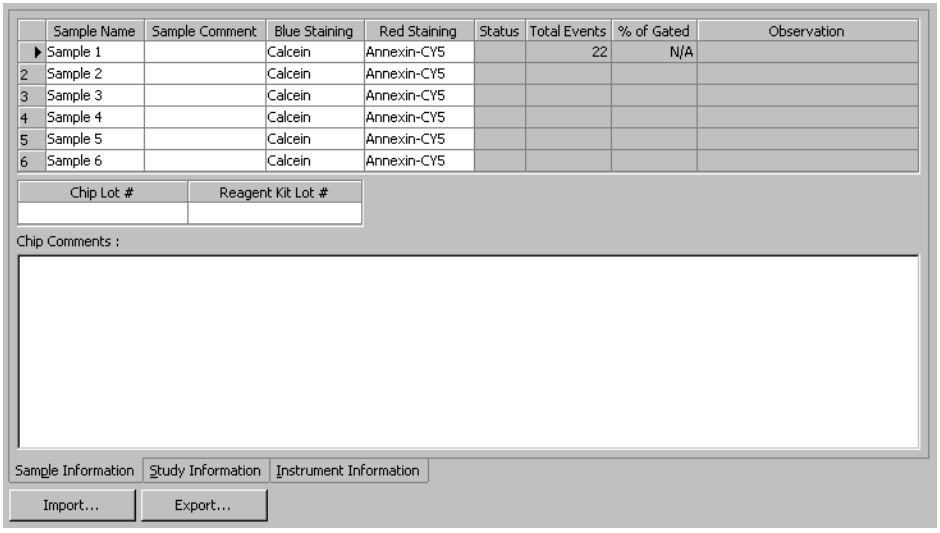
2 Accept the current *File Prefix* or modify it.

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 as dot plot 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 online dot plot, return to the Instrument context.

6 After the chip run is finished remove the chip from the receptacle of the Agilent 2100 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.

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

Protocol Modifications for On-Chip Staining

NOTE For a detailed on-chip staining protocol, please refer to the appropriate on-chip staining application note (http://www.agilent.com/chem/labonachip).

1. Prime the chip like described in "Loading the Chip Priming Solution" on page 13.
2. Add 10 µl of focusing dye solution (● yellow) and 30 µl of cell buffer (green) like described in "Loading the Focusing Dye Solution, Cell Buffer and Samples" on page 14.
3. Add 10 µl cell suspension and 4 µl of the required staining reagents in each sample well. Pipette a total of 14 µl of liquid into the sample wells. If less than 6 samples are analyzed, pipette 14 µl of cell buffer (● green) in any empty sample well.
4. Mix the cell suspension with the staining reagent by vortexing the chip for 1 minute. Use the IKA vortexer supplied with the Agilent 2100 bioanalyzer and adjust the speed knob to the 12 o'clock position.

5 The recommended cell concentration for the 10 µl of cell suspension in CB buffer used with on-chip staining is 3.0 million cells/ml.

6 To stain the cells, incubate the prepared chips for the time specified in the application note at room temperature. Incubate in a humidified chamber or staple chips to prevent excessive evaporation.

NOTE If a new cell line or staining reagents are used, incubation times may need optimization.

7 Resuspend the cells after the incubation step by vortexing the chip again for 1 minute like described above.

Checking Your Agilent Cell Assay Results

Cell Assay Results

To check the results of your run, select the *Data and Assay* context.
To check the results of a specific sample, select the sample name in the tree view and highlight the *Histogram* or *Dot-Plot* tab. The histograms and the dot blots should resemble the ones shown here.

For more information on evaluating flow cytometric assays please see detailed description within the *2100 Expert help files*.

NOTE

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

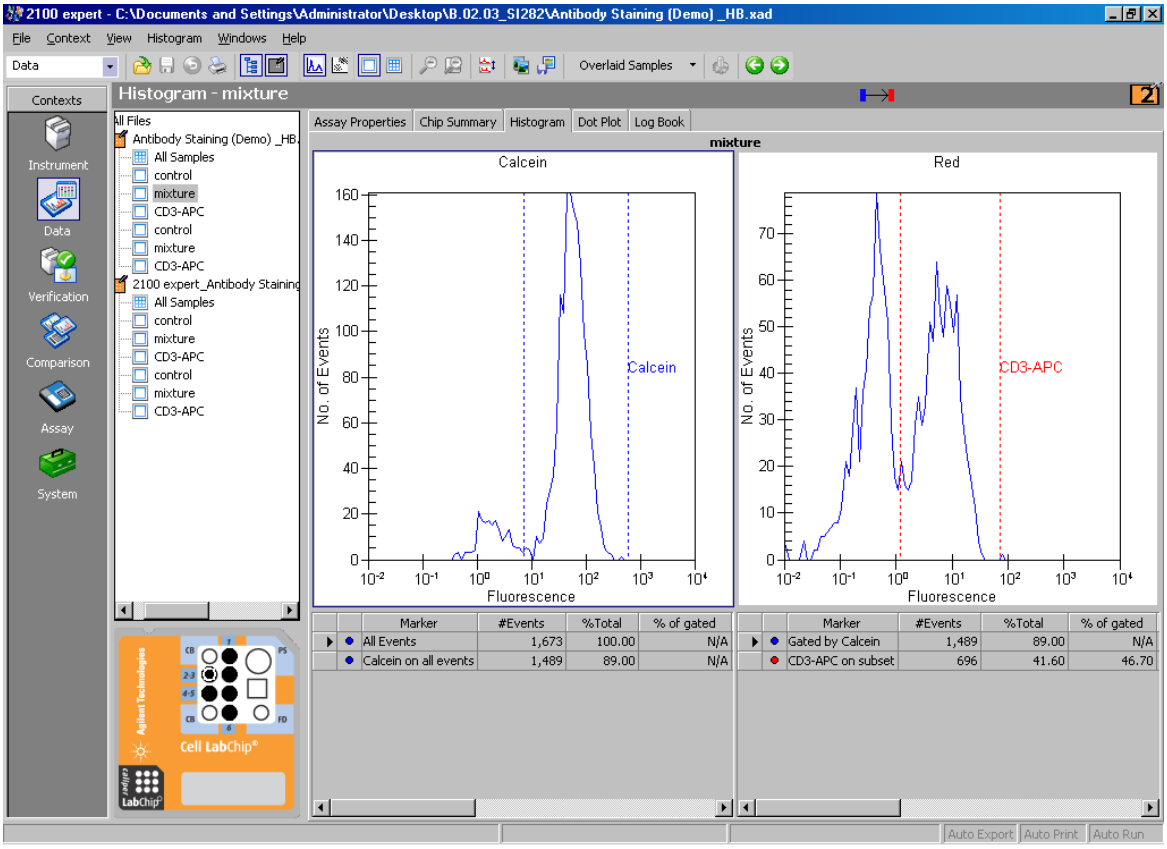


Figure 1 Cell assay histogram, example

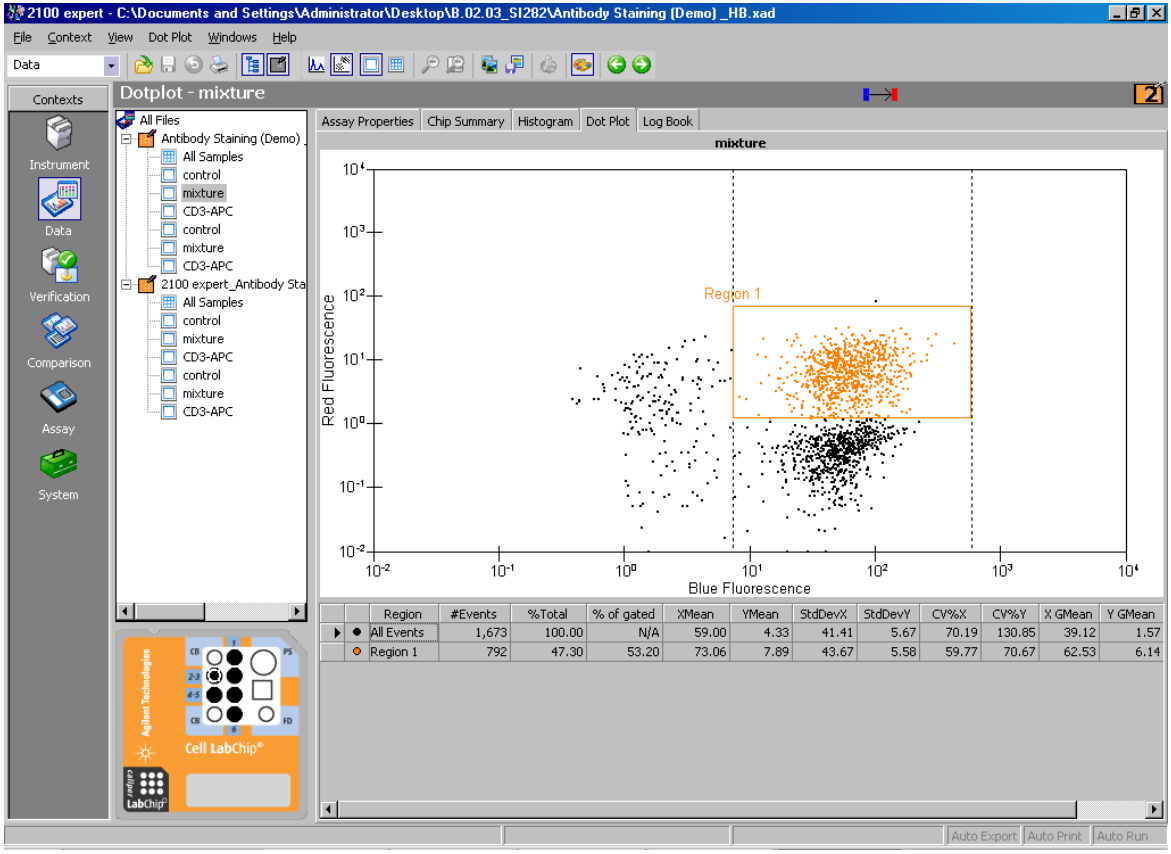


Figure 2 Cell assay dot-plot, example

Index

www.agilent.com

In This Book

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

Printed in Germany
06/06

G2938-90072

Agilent Technologies