Notices

© Agilent Technologies, Inc. 2000-2003

No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent Technologies, Inc. as governed by United States and international copyright laws.

Part number G2938-90050
Printed in Germany

Agilent Technologies, Deutschland GmbH
Hewlett-Packard-Strasse 8
76337 Waldbronn

Trademarks and Technology Licenses
Adobe and Acrobat are U.S. registered trademarks of Adobe Systems Incorporated.
Microsoft® and Windows® are U.S. registered trademarks of Microsoft Corporation.

Caliper®, LabChip® and the LabChip logo are registered trademarks of Caliper Technologies Corp. in the U.S. and other countries.

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.

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.

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

Protein 200 Plus Assay Kit ..................................................................................................... 5

Equipment Required for a Protein Assay .............................................................................. 6

Setting up the Assay Equipment and Bioanalyzer ............................................................... 7
  Setting up the Chip Priming Station .................................................................................. 8
  Setting up the Bioanalyzer ............................................................................................... 9
  Starting the 2100 Expert Software .................................................................................. 10

Essential Measurement Practices ...................................................................................... 12

Protein 200 Plus Assay Protocol ......................................................................................... 14
  Preparing the Gel-Dye Mix and Destaining Solution ......................................................... 15
  Preparing the Denaturing Solution .................................................................................. 18
  Preparing the Samples and the Ladder .......................................................................... 19
  Loading the Gel-Dye Mix ................................................................................................. 21
  Loading the Samples and the Ladder ............................................................................. 23
  Inserting the Chip in the Agilent 2100 Bioanalyzer ......................................................... 24
  Starting the Chip Run ...................................................................................................... 26
  Cleaning Electrodes After a Chip Run ............................................................................. 29
Contents

- Checking Your Protein 200 Plus Results ................................................................. 30
  - Protein 200 Plus Ladder Well Results ................................................................. 30
  - Protein Sample Well Results .............................................................................. 33
- List of Compatible Buffers and Buffer Compounds ............................................. 37
- Protein 200 Plus Assay Quick Reference Guide
Protein 200 Plus Assay Kit

Make sure that your Protein 200 Plus LabChip® kit comes with the following items:

**Protein 200 Plus LabChip® Kit (reorder number 5065-4480)**

**Protein Chips**
- 25 Protein Chips
- 1 Electrode Cleaner

**Protein 200 Plus Reagents & Supplies**
- ● Protein 200 Plus Gel-Matrix (4 vials)
- ● Protein 200 Plus Dye Concentrate*
- ○ Protein 200 Plus Sample Buffer (4 vials)
- ● Protein 200 Plus Ladder
- 4 Spin Filters

**Syringe Kit**
- 1 Syringe

* Protein dye concentrate is manufactured by Molecular Probes, Inc. and licensed for research use only

Check [www.agilent.com/chem/labonachip](http://www.agilent.com/chem/labonachip) for new details on assays.
Equipment Required for a Protein Assay

Equipment supplied with the Agilent 2100 bioanalyzer

- Chip Priming Station (reorder number 5065-4401)

Additional material required

- Pipettes (10 µl, 20 µl, 100 µl and 1000 µl) with compatible tips
- 0.5 ml microcentrifuge tubes
- Deionized water
- β-mercaptoethanol (BME) or dithiothreitol (DTT)
- Microcentrifuge
- Heating block for 0.5 ml tubes or water bath
- Vortexer
Setting up the Assay Equipment and Bioanalyzer

Before beginning the chip preparation protocol, ensure that the Chip Priming Station and the bioanalyzer are set up and ready to use.

You have to

• replace the syringe at the Chip Priming Station with each new kit
• adjust the base plate of the Chip Priming Station
• adjust the syringe clip at the Chip Priming Station, and
• adjust the bioanalyzer’s chip selector
• Finally, make sure you start the bioanalyzer software before you load the chip.
Setting up the Chip Priming Station

NOTE
Replace the syringe with each new Reagent Kit.

1 Replace the syringe:
   a) Unscrew the old syringe from the lid of the Chip Priming Station.
   b) Release the old syringe from the clip. Discard the old syringe.
   c) Insert the new syringe into the clip.
   d) Slide it into the hole of the luer lock adapter and screw it tight.

2 Adjust the base-plate:
   a) Open the Chip Priming Station by pulling the latch.
   b) Using a screwdriver, open the screw at the underside of the base plate.
   c) Lift the base-plate and insert it in position A. Retighten the screw.
3 Adjust the syringe clip:
Release the lever of the clip and slide it down to the middle position.

**Setting up the Bioanalyzer**

Adjust the chip selector:

1. Open the lid of the bioanalyzer and make sure that the electrode cartridge is inserted in the instrument. If not, open the latch, remove the pressure cartridge and insert the electrode cartridge.

2. Remove any remaining chip and adjust the chip selector to position (1).
Starting the 2100 Expert Software

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, Protein 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.
Essential Measurement Practices

- Handle and store all reagents according to the instructions.
- 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.
- Store Protein 200 Plus sample buffer at -20 °C upon arrival. Keep the vial in use at 4 °C to avoid freeze-thaw cycles.
- Allow the dye concentrate to equilibrate to room temperature for 30 minutes before use, to make sure the DMSO is completely thawed. Protect the dye from light during that time. Vortex before use.
- Allow all other reagents to equilibrate to room temperature for 10 minutes before use.
- Protect dye concentrate and gel-dye mix from light. Remove light covers only when pipetting. The dye decomposes when exposed to light.
- 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.
- Use a new syringe and a new electrode cleaner with each new LabChip® kit.
• Use 0.5 ml tubes to denature samples. Using larger tubes may lead to poor results, caused by evaporation.

• Use loaded chip within 10 minutes. Reagents may evaporate, leading to poor results.

• Do not touch the Agilent 2100 bioanalyzer during an assay run and never place it on a vibrating surface.
Protein 200 Plus Assay Protocol

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

**NOTE**

If you use the Protein 200 Plus LabChip® kit for the first time, you must read these detailed instructions. If you have some experience, you might want to use the *Protein 200 Plus Assay Quick Reference Guide* at the end of this guide.
Preparing the Gel-Dye Mix and Destaining Solution

**WARNING**
Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.
Kit components contain DMSO, SDS, and LDS. No data is available addressing the mutagenicity or toxicity of the dye/DMSO reagent. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solutions should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

1. Take a vial of Protein 200 Plus dye concentrate (blue ●) from the refrigerator and allow it to equilibrate to room temperature for 30 min.

   ![gel-dye mix](image)

   - 25 µl dye
   - 650 µl gel

**NOTE**
It is important that the reagents all have room temperature before starting the next step. Protect the dye concentrate from light when not in use.

2. Vortex well (10 seconds at highest setting) and spin down for 15 seconds. Make sure the DMSO is completely thawed.
3 Pipette 25 µl from the Protein 200 Plus dye concentrate (blue ●). Open a tube of Protein 200 Plus gel matrix (red ●) and transfer the 25 µl of dye concentrate.

**NOTE**
Always use the volumes indicated. Using different volumes in the same ratio will produce inaccurate results.

4 Cap the tube, vortex well (10 seconds at highest setting) and spin down for 15 seconds.

5 Store the dye concentrate in the dark again.

6 Open the tube and transfer the gel-dye mix to the top receptacle of a spin filter. Label the tube and include the date of preparation.

**NOTE**
The gel-dye mix is sufficient for 9 chips. Use the gel-dye mix within four weeks of preparation, and protect it from light at all times.
Store the gel-dye mix at 4 °C when not in use for longer than one hour.

7 To produce the destaining solution open another tube of gel matrix (red ●).

8 Transfer the gel into the top receptacle of a new spin filter and label the tube.
9 Place the spin filters with the gel-dye mix and the destaining solution in a microcentrifuge and spin for 15 minutes at room temperature at 2500 g ± 20 % (for Eppendorf microcentrifuge, this corresponds to 5200 rpm).

10 Discard the filter according to good laboratory practices.

**NOTE**

The prepared destaining solution is sufficient for 25 chips and is stable for the complete kit lifetime.

Store the destaining solution at 4 °C when not in use for longer than one hour.
Preparing the Denaturing Solution

1. Remove one vial of Protein 200 Plus sample buffer (white) from freezer. Allow to equilibrate to room temperature for 10 minutes, then vortex.

2. For reducing conditions, add 7 µl of ß-mercaptoethanol (BME) or 7 µl of dithiothreitol (1 M) to the 200 µl sample buffer in the original vial.
   For non-reducing conditions, add 7 µl of deionized water to 200 µl sample buffer.
   
   **NOTE**
   Adjust volumes to prepare smaller batches.

3. Vortex for 5 seconds.

   **NOTE**
   This denaturing solution is sufficient for 10 chips. Use the prepared denaturing solution within four weeks.
   To avoid freeze thaw cycles store the denaturing solution as well as smaller batches of sample buffer at 4 °C when not in use for longer than 1 hour.
Preparing the Samples and the Ladder

NOTE
For a list of compatible buffers, please refer to the chapter “List of Compatible Buffers and Buffer Compounds” on page 37.

1 Allow the denaturing solution (prepared as described in “Preparing the Denaturing Solution” on page 18) and the Protein 200 Plus ladder vial (yellow ●) to equilibrate to room temperature for 10 min, and vortex before use.

2 Combine 4 µl of your protein sample and 2 µl of denaturing solution in a 0.5 ml microcentrifuge tube. Using larger tubes may lead to poor results.

3 Mix well and spin down for 15 seconds.

4 Pipette 6 µl of ladder in a 0.5 ml microcentrifuge tube (do not add denaturing solution).

5 Place each sample tube and the ladder tube for 3 to 5 minutes in a heating block at 95-100 °C or in boiling water.

   Ensure that the tubes are properly placed and heated. Do not heat for longer than 5 minutes or excessive evaporation may occur. The samples and ladder should not dry down.

6 Let the tubes cool down for 10 seconds and spin them for 15 seconds.
To each sample and ladder tube add 84 µl of deionized water and vortex.

It is not recommended to change the dilution ratio. This will not improve sensitivity, but might lead to poor results and quantitation errors.

**NOTE**

The diluted samples and ladder are stable for one day. Store samples at 4 °C when not in use for longer than 1 hour. For your convenience you might want to prepare twenty five 6 µl aliquots of ladder (amount needed for one chip) and store them at 4 °C.
Loading the Gel-Dye Mix

1. Allow the gel-dye mix and the destaining solution to equilibrate to room temperature for 10 minutes before use. Protect the gel-dye mix from light during this time.

2. Take a new protein chip out of its sealed bag.

3. Place the chip on the Chip Priming Station. Make sure it is set-up as described in “Setting up the Assay Equipment and Bioanalyzer” on page 7.

4. Pipette 12 µl of the gel-dye mix in the well marked ⬆️. Insert the tip of the pipette to the bottom of the well when dispensing. Placing the pipette at the edge of the well may lead to poor results.

5. Set the timer to 60 seconds. Make sure that the plunger is at 1 ml, then close the Chip Priming Station.
6 Press the plunger until it is held by the syringe clip.

7 Wait for exactly 60 seconds and then release the plunger with the clip release mechanism.

8 Wait for 5 seconds, then slowly pull back the plunger to the 1 ml position.

9 Open the Chip Priming Station.

10 Remove and discard the remaining solution in the well marked 6.

11 Pipette 12 µl of the gel-dye mix in each of the 4 wells marked 6 and 6.

12 Pipette 12 µl of the destaining solution in the well marked 6.
Loading the Samples and the Ladder

1 Pipette 6 µl of the diluted samples (prepared as described in “Preparing the Samples and the Ladder” on page 19) into the sample wells marked 1...10.

Insert the tip of the pipette to the bottom of the well when dispensing. Placing the pipette at the edge of the well may lead to poor results.

2 Pipette 6 µl of the diluted ladder into the ladder well 🚷.

NOTE
Do not leave any wells empty, or the chip will not run properly. Pipette 6 µl of sample or ladder replicate in any empty sample well.

3 Start the run immediately. See next page on how to place the chip in the Agilent 2100 bioanalyzer.
Inserting the Chip in the Agilent 2100 Bioanalyzer

1. Open the lid of the Agilent 2100 bioanalyzer. Check that the electrode cartridge is inserted properly and the chip selector is in position (1).

2. Place the chip into the receptacle. The chip fits only one way. Do not use force.

3. Carefully close the lid. The electrodes in the cartridge fit into the wells of the chip.

**CAUTION**
Do not force the lid closed. Otherwise electrodes may be damaged.

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

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.

3. Click the *Start* button in the upper right of the window to start the chip run. The raw data is displayed in the *Instrument* context.
During data acquisition the sample information can be completed: 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 table and press Apply.

If absolute quantitation is required with a standard protein, mark the check box Use of calibration and enter standard concentration.

To review the raw signal trace, return to the Instrument context.
6 When the chip run is complete, the *End of Run* message appears.

![End of Run message](image)

7 Remove the chip from the receptacle of the Agilent 2100 bioanalyzer and dispose it according to good laboratory practice.

**CAUTION**

Immediately remove the chip after a run. Leaving it for a longer period than 1 hour in the bioanalyzer may cause contamination of electrodes.
Cleaning Electrodes After a Chip Run

When the assay is complete, *immediately* remove the used chip from the Agilent 2100 bioanalyzer and dispose 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).

1. Slowly fill one of the wells of the electrode cleaner with 350 µl 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.

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 to DNA or RNA assays, a more thorough cleaning may be required. Refer to the *2100 Expert Users Guide* for details.
Checking Your Protein 200 Plus Results

To review the results of a chip run, select the Data and Assay context.

Data is displayed as electropherogram or gel-like image. To check the results of a specific sample, select the sample name in the tree view and highlight the Results or Peak Table sub-tab.

Protein 200 Plus Ladder Well Results

To check the results of your run, go to the Data and Assay context and select the ladder from the Tree View. The electropherogram of the ladder well window should resemble the one shown here.
Major features of a successful ladder run are:

- 11 ladder peaks and all peaks are well resolved
- flat baseline
- readings at least 20 fluorescence units higher than baseline readings.

If your electropherogram of the ladder well window does not resemble the one shown above, refer to the *2100 Expert Maintenance & Troubleshooting Guide*.

In some of your runs, you might see a double system peak, as shown below. Usually this can be handled by the software and does not cause a problem.
In case both system peaks are identified as ladder peaks, exclude peak 2 (the left of the two peaks) by doing the following:

1. Move the cursor over the second peak in the peak table and click the right mouse button.
2 Select *Exclude Peak from Ladder* to make the change come into effect.

**Protein Sample Well Results**

If you are not viewing your results in single well mode, select one of the sample in the *Tree View* and highlight the *Electropherogram* tab in the *Data and Assay* context. The electropherogram of the sample well window should resemble the one shown here for the Protein 200 Plus assay.

![Electropherogram](image)

Major features for a successful sample run are:

- all sample peaks between the lower and upper marker peaks
- two marker peaks, one system peak
- lower marker peak between 16 and 23 seconds
• upper marker peak between 36 and 43 seconds
• baseline readings between 100 and 300 fluorescence units (to enable the “zero baseline” setting see Zero Baseline in the 2100 Expert User’s Guide or Online Help)
• both marker peaks well resolved from sample peaks (depending on sample)
• The upper marker is 95 % pure and contains two small impurities at 18 and 25 kDa. The impurity level of the upper marker is corrected by the software in the concentration determination.

NOTE
Baseline correction can affect quantitation when analyzing broad peaks (e.g. non-reduced IgG or cell lysates) and should be turned off for accurate quantitation.

In some of your runs, you might see a shoulder, or a small peak on the left of the lower marker.
As this is not a lower marker peak, make sure that it is not treated as a lower marker, otherwise sizing will be affected.

To do this, use the following procedure:

1. Select the *Peak table* sub-tab and move the cursor over the second peak listed in the table.
2. Click the right mouse button.
3. Select *Manually set lower marker.*
Data is now re-analyzed with the selected peak set as lower marker.

For easier identification of the correct lower and upper marker, turn off the alignments to identify and manually assign markers. To turn the alignment off, select Gel > Don’t analyze. Compare markers in samples to markers in ladder by following the drift in the gel-like image.

For troubleshooting, please refer to the 2100 Expert Maintenance & Troubleshooting Guide.
# List of Compatible Buffers and Buffer Compounds

The following tables list protein sample buffers and buffer components which are known to be compatible with the Protein 200 Plus LabChip® kit.

For an updated list please refer to the web-site [www.agilent.com/chem/labonachip](http://www.agilent.com/chem/labonachip).

## Salts and Buffers

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Concentration</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM HEPES</td>
<td>pH 7.5</td>
<td></td>
</tr>
<tr>
<td>25 mM HEPES / 150 mM NaCl</td>
<td>pH 7.5</td>
<td></td>
</tr>
<tr>
<td>20 mM HEPES / 20% glycerol / 0.1 M KCl / 0.2 mM EDTA / 0.5 mM PMSF / 0.5 mM DTT</td>
<td>pH 7.9</td>
<td></td>
</tr>
<tr>
<td>20 mM Histidine / 30 mM NaCl / 30 mM sucrose / 290 mM glycine / 2 mM CaCl₂</td>
<td>pH 6.6</td>
<td></td>
</tr>
<tr>
<td>250 mM imidazole in PBS</td>
<td>pH 7.4</td>
<td></td>
</tr>
<tr>
<td>300 mM KCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mM MES</td>
<td>pH 6.0</td>
<td></td>
</tr>
<tr>
<td>5 mM MgCl₂O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM MOPS</td>
<td>pH 7.2</td>
<td></td>
</tr>
<tr>
<td>20 mM Na acetate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 mM NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salts and Buffers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mM NaF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 mM NH₄HCO₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 mM NH₄SO₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mM Na or K phosphate pH 7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mM Na phosphate / 15 mM NaCl pH 7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mM NaH₂PO₄ / 300 mM NaCl / 250 mM imidazole / 2 mM Na pyrophosphate / 1 mM Na orthovanadate / 5 mM BME / 0 - 500 mM imidazole pH 7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 mM NaH₂PO₄ / 41 mM Na₂HPO₄ / 79 mM NaCl pH 8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS pH 7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mM PIPES pH 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM Tris-HCl pH 8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM Tris-bis-propane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM Tris / 3 mM desthiobiotin pH 8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250 mM Tris / 20 mM glycine pH 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM Tris/150 mM Na citrate pH 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mM Tris / 500 mM NaCl / 0 - 500 mM imidazole pH 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 mM Tris / 22.5 mM NaCl / 10 % glycerol pH 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mM Tris / 500 mM NaCl / 25 mM β-glycerophosphate pH 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mM Tris / 10 mM glutathione pH 8.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The numbers to the right indicate effects that may occur if that particular buffer is used:

(1) gives large system peak which overlaps with lower marker, slightly affects sizing
(2) upper marker decreased, quantitation affected
(3) at higher concentrations: lower marker disappears, negative dip between 9-20 kDa, no sizing possible
(4) negative dip between 14-19 kDa

<table>
<thead>
<tr>
<th>Detergents</th>
<th>Possible Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 % CHAPS in PBS pH 7.4</td>
<td>(1, 2)</td>
</tr>
<tr>
<td>0.1 % desoxycholate in PBS pH 7.4</td>
<td>(3)</td>
</tr>
<tr>
<td>2 % dodecyl beta maltoside in PBS pH 7.4</td>
<td>(1)</td>
</tr>
<tr>
<td>1 % sarcosyl in PBS pH 7.4</td>
<td>(2, 4)</td>
</tr>
<tr>
<td>1 % SDS</td>
<td></td>
</tr>
<tr>
<td>1 % Triton X-100</td>
<td>(1)</td>
</tr>
<tr>
<td>0.1 % Tween 20</td>
<td>(1)</td>
</tr>
<tr>
<td>0.5 % zwittergent E3-14 in PBS pH 7.4</td>
<td>(1, 2)</td>
</tr>
</tbody>
</table>

Other additives                               Possible Effects

<table>
<thead>
<tr>
<th>Other additives</th>
<th>Possible Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 % acetonitrile</td>
<td>(5)</td>
</tr>
<tr>
<td>10 % DMSO</td>
<td></td>
</tr>
<tr>
<td>100 mM DTT</td>
<td></td>
</tr>
<tr>
<td>Other additives</td>
<td>Possible Effects</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>10 mM EDTA</td>
<td></td>
</tr>
<tr>
<td>20 % ethanol</td>
<td></td>
</tr>
<tr>
<td>600 mM guanidine</td>
<td>(6)</td>
</tr>
<tr>
<td>1 mM HCl</td>
<td></td>
</tr>
<tr>
<td>5 % mannitol</td>
<td></td>
</tr>
<tr>
<td>0.04 % NaN₃</td>
<td></td>
</tr>
<tr>
<td>30 % glycerol</td>
<td></td>
</tr>
<tr>
<td>1 % PEG 2000 (polyethylene glycol)</td>
<td></td>
</tr>
<tr>
<td>Protease inhibitor cocktail (100x diluted, Sigma)</td>
<td></td>
</tr>
<tr>
<td>200 mM sucrose</td>
<td></td>
</tr>
<tr>
<td>0.1 % TFA</td>
<td>(7)</td>
</tr>
<tr>
<td>7 M urea in PBS</td>
<td></td>
</tr>
<tr>
<td>0.01 % Pluronic F68 (contained in lipid concentrates used in cell culture)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

(5) can precipitate SDS, must be evaporated, quantitation might be affected
(6) precipitates SDS, quantitation might be affected
(7) acidic buffers might affect sizing, must be evaporated or neutralized
(8) causes a peak at 22-24 kDa at higher concentrations interfering with other proteins in this size range. If above 5 %, the lower and upper marker are affected.
## Index

### Numerics
- 2100 expert software, 10, 24

### A
- assay principles, 43

### B
- base-plate, 8
- bioanalyzer, 9

### C
- chip, 24
- chip priming station, 6, 8, 21
- chip selector, 9, 24
- cleaning, 29
- compatible buffers, 37

### D
- deionized water, 6, 20
- denaturing solution, 18
- destaining solution, 15, 22
- detergents, 39
- dithiothreitol, 6, 18
- DMSO, 12
- dye concentrate, 5, 12, 15

### E
- electrode cartridge, 9, 24
- electrode cleaner, 5, 29
- essential measurement practices, 12

### F
- file prefix, 26

### G
- gel, 5
- gel-dye, 15, 21

### H
- heating block, 6

### I
- instrument context, 10, 24

### L
- ladder, 5, 19, 23
- ladder electropherogram, 30
loading
destaining solution, 22
gel-dye, 21
ladder, 23
sample, 23

M
marker, 33
mercaptoethanol, 6, 18
microcentrifuge, 6
microcentrifuge tubes, 6

P
plunger, 21
preparation
denaturing solution, 18
destaining solution, 15
gel-dye, 15
ladder, 19
sample, 19
protocol, 14, 44

Q
quantitation, 27
quick reference, 43

R
reagents & supplies, 5

results, 30

S
salts, 37
sample, 19, 23
sample buffer, 5, 12, 18
sample electropherogram, 33
sample information, 27
set up
base-plate, 8
bioanalyzer, 9
chip priming station, 8
syringe clip, 9
specifications
analytical, 44
physical, 43
spin filters, 5
storage
denaturing solution, 18
destaining solution, 17
gel-dye, 16
samples, 20
storage conditions, 43
support, 43
syringe, 5, 8
syringe clip, 9
Protein 200 Plus Assay Quick Reference Guide

Assay Principles
LabChip® kits contain chips and reagents designed for sizing and analysis of proteins. Each chip contains an interconnected set of microchannels that sieve proteins by size as they are driven through it by means of electrophoresis. LabChip® kits are designed for use with the Agilent 2100 bioanalyzer only.

Assay Kit
The Protein 200 Plus LabChip® kit is designed for the sizing and analysis of proteins from 14-200 kDa and can be used to analyze cell lysates, column fractions or purified proteins.

Storage Conditions
- Keep all reagents and reagent mixes at the indicated temperature when not in use to avoid poor results caused by reagent decomposition.
- Store Protein 200 Plus sample buffer at -20 °C upon arrival. To avoid freeze-thaw cycles, the vial in use should be stored at 4 °C.
- Protect dye and gel-dye mix from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.

Other Protein Kits
- Protein 50 Kit (reorder number 5065-4484)
- Protein 50 Reagents & Supplies (reorder number 5065-4485)
- Protein 200 Plus Reagents & Supplies (reorder number 5065-4482)

Protein 200 Plus Assay - Physical Specifications

<table>
<thead>
<tr>
<th>Type</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis run time</td>
<td>25 minutes</td>
</tr>
<tr>
<td>Number of samples</td>
<td>10 samples/chip</td>
</tr>
<tr>
<td>Sample volume</td>
<td>4 µl</td>
</tr>
<tr>
<td>Assay kit stability</td>
<td>3 months at indicated temperature</td>
</tr>
</tbody>
</table>

Additional Materials and Equipment (not supplied)
- Pipettes (10 µl, 20 µl, 100 µl, and 1000 µl) with compatible tips
- 0.5 ml microcentrifuge tubes
- Deionized water
- Mercaptoethanol (BME) or dithiothreitol (DTT)
- Microcentrifuge
- 0.5 ml heating block or water bath
- Vortexer

Technical Support:
In the U.S./Canada 1-800-227-9770 (toll free)
bioanalyzer_americas@agilent.com
In Europe bioanalyzer_europe@agilent.com
In Japan 0120 477 111
lab_chip@agilent.com
In Asia Pacific (+81) 422 56 93 92
bioanalyzer_ap@agilent.com

Further Information
Visit Agilent Technologies’ unique Lab-on-a-Chip web site offering useful information, support and current developments about the products and technology: http://www.agilent.com/chem/labonachip
**Essential Measurement Practices**

- Always insert the pipette tip into the bottom of the well when dispensing liquids. Placing the pipette at the edge of the well may lead to poor results.
- Store Protein 200 Plus sample buffer at -20 °C upon arrival. Keep the vial in use at 4 °C to avoid freeze-thaw cycles.
- Allow the dye concentrate to equilibrate to room temperature for 20 minutes before use. Other reagents should equilibrate to room temperature for 10 minutes.
- Protect dye and gel-dye mix from light. Remove light covers only when pipetting.
- Use 0.5 ml tubes to denature samples. Using larger tubes may lead to poor results.
- Use loaded chip within 10 minutes. Reagents may evaporate.
- Do not touch the Agilent 2100 bioanalyzer during an assay run and never place it on a vibrating surface.
- Avoid sources of dust or other contaminants.

**Protein 200 Plus Analytical Specifications**

<table>
<thead>
<tr>
<th>Type</th>
<th>Protein 200 Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sizing range</td>
<td>14-200 kDa</td>
</tr>
<tr>
<td>Sizing resolution</td>
<td>± 10 % across size range</td>
</tr>
<tr>
<td>Concentration range</td>
<td>sensitivity equivalent to non-colloidal coomassie stain, e.g. 20–2000 ng/µl BSA in PBS</td>
</tr>
<tr>
<td>Compatible buffers</td>
<td>see “List of Compatible Buffers and Buffer Compounds” on page 37</td>
</tr>
</tbody>
</table>


**Preparation Gel-Dye Mix**

1. Add 25 µl of protein 200 plus dye concentrate (blue) to protein 200 plus gel matrix (red) tube. Vortex well and spin down the tube for 15 s.
2. Transfer to spin filter.
3. Centrifuge at 2500 g ± 20 % for 15 min.

**Destaining Solution**

1. Pipette 650 µl of gel matrix (red) into spin filter.
2. Centrifuge at 2500 g ± 20 % for 15 min. One tube is sufficient for 1 kit (25 chips).

**Preparing Denaturing Solution**

1. Add 7 µl of β-mercaptoethanol or dithiothreitol (1 M) to the sample buffer vial (200 µl, white).
2. Vortex for 5 s.

**Preparing the Samples and the Ladder**

1. Combine 4 µl protein sample and 2 µl denaturing solution in 0.5 ml tube.
2. Place sample tubes and tube with 6 µl protein 200 plus ladder (yellow) at 100°C for 5 min.
3. Spin tubes for 15 s.
4. Add 84 µl deionized water to samples and ladder and vortex.

**Loading the Gel-Dye Mix**

1. Put a new protein chip on the Chip Priming Station.
2. Pipette 12 µl of gel-dye mix in the well marked G.
3. Put plunger at 1 ml and close Chip Priming Station.
4. Press plunger until held by clip, wait 60 s, then release clip.
5. Remove solution in well G.
6. Pipette 12 µl of gel-dye mix in G and DS.
7. Pipette 12 µl of destaining solution in well DS.

**Loading the Samples and the Ladder**

1. Pipette 6 µl of sample in 10 sample wells.
2. Pipette 6 µl of the ladder in the well marked G.
3. Place the chip in the Agilent 2100 bioanalyzer and start immediately.

**WARNING** — Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. DMSO is known to facilitate the entry of organic molecules into tissues.