Agilent Protein 80 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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Contents

Agilent Protein 80 Kit

Protein 80 Kit (reorder number 5067-1515)	
Protein Chips	**Protein 80 Reagents (reorder number 5067-1516) & Supplies**
25 Protein Chips	● (red) Protein 80 Gel-Matrix (4 vials) in box labelled Part I. Store at 4 °C
1 Electrode Cleaner	● (blue) Protein 80 Dye Concentrate* in box labelled Part I. Store at 4 °C
	❍ (white) Protein 80 Sample Buffer (4 vials) in box labelled Part II. Store at -20 °C
Syringe Kit	● (yellow) Protein 80 Ladder in box labelled Part II. Store at -20 °C
1 Syringe	4 Spin Filters

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Physical Specifications	
Type	**Specification**
Analysis run time	30 minutes
Number of samples	10 samples/chip
Sample volume	4 µl
Assay Kit stability	4 months (storage temperature see individual box)

CAII	= Carbonic Anhydrase
BSA	= Bovine Serum Albumine
BLG	= beta-Lactoglobulin

Analytical Specifications	
Type	**Agilent Protein 80 Assay**
Sizing range	5-80 kDa
Typical sizing resolution	10 %
Typical sizing accuracy	10 % CV (CAII, BLG)
Sizing reproducibility	3 % CV (CAII, BLG)
Sensitivity (Signal/Noise>3)	6 ng/µl CAII (15 ng/µl BSA) in PBS, 10 ng/µl (CAII) in 0.5 M NaCl (30ng/µl BSA in 0.5 M NaCl)
Quantitative range	60-2000 ng/µl CAII in PBS
Qualitative range	6-4000 ng/µl CAII and BLG in PBS
Quantitation reproducibility	20 % CV (CAII, BLG)
Compatible buffers	see "List of Compatible Buffers and Buffer Compounds" on page 29

Equipment Required for a Protein 80 Assay

Equipment supplied with the Agilent 2100 bioanalyzer

- Chip Priming Station (reorder number 5065-4401)

Additional material required (not supplied)

- Pipettes (10 µl, 20 µl, 100 µl and 1000 µl) with compatible tips
- 0.5 ml microcentrifuge tubes
- Deionized water
- 1 M Dithiothreitol (DTT) solution or ß-mercaptoethanol (BME)
- Microcentrifuge
- Heating block for 0.5 ml tubes or water bath

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 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 Protein kit
- adjust the base-plate of the Chip Priming Station
- adjust the syringe clip at the Chip Priming Station
- adjust the bioanalyzer's chip selector
- Finally, make sure that you start the software before you load the chip.

NOTE The Agilent Protein 80 assay is a high sensitivity assay. Please read this guide carefully and follow all instructions to guarantee satisfactory results.

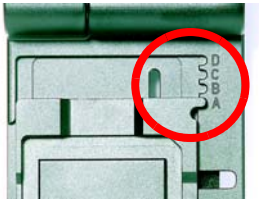
Setting up the Chip Priming Station

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** Remove the plastic cap of the new syringe and insert it into the clip.
- **d** Slide it into the hole of the luer lock adapter and screw it tightly to the Chip Priming Station.

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 again in position A. Retighten the screw.

3 Adjust the syringe clip:

- **a** Release the lever of the clip and lift it up or down to adjust it to the **middle** position.

Setting up the Bioanalyzer

Adjust the chip selector:

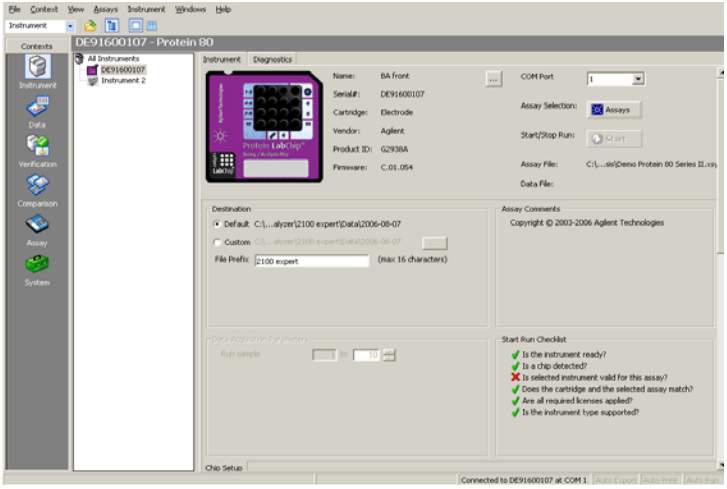
1. Open the lid of the bioanalyzer and make sure that the electrode cartridge is inserted in the instrument. If not, open the latch, remove the pressure cartridge and insert the electrode cartridge.
2. Remove any remaining chip and adjust the chip selector to position (1).

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

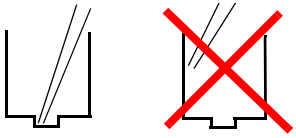
Dimmed icon: no communication

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 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.
- Upon arrival make aliquots for the sample buffer and the ladder with the typical amount required for daily use and store them at -20 °C. Keep the vial in use at 4 °C to avoid freeze-thaw cycles.
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes before use.
- Protect all reagents from light. Remove light covers only when pipetting. The dye contained in the reagents 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.



- Use a new syringe and electrode cleaners with each new kit.
- Use loaded chips within 5 minutes. Reagents might evaporate, leading to poor results.
- Do not touch the Agilent 2100 bioanalyzer during analysis and never place it on a vibrating surface.
- Use 0.5 ml vials to denature samples. Using larger vials may lead to poor results, caused by evaporation.

Agilent Protein 80 Assay Protocol

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

Preparing the Gel-Dye Mix

WARNING

Handling DMSO

Kit components contain DMSO/dye. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care.

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

⇒ Handle the DMSO/dye solutions with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

1 Allow the Protein 80 Dye Concentrate ● (blue) and the Protein 80 Gel-Matrix ● (red) to equilibrate to room temperature for 30 minutes.

NOTE

It is important that all the reagents have room temperature before starting the next step. Protect the Dye Concentrate from light.

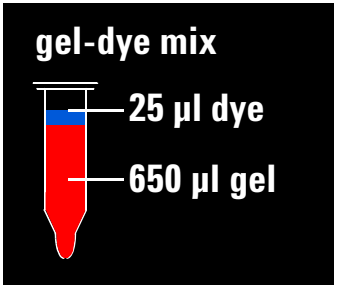
2 Transfer the content (650 µl) of an Agilent Protein 80 Gel-Matrix vial ● (red) to a spin filter. Make sure the complete volume of 650 µl has been transferred.

3 Centrifuge at 2500 g ± 20 % for 15 min.

4 To the filtered and centrifuged Gel add 25 µl of the well vortexed Dye Concentrate ● (blue).

5 Mix thoroughly for 10-20 s (Vortexer) until a uniform color is obtained.

6 Label with the date and GD (Gel/Dye).



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

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 more than one hour.

Preparing the Destaining Solution

1 Transfer the content (650 µl) of another Protein 80 Gel-Matrix vial ● (red) to a spin filter. Make sure the complete volume of 650 µl has been transfered.

2 Centrifuge at 2500 g ± 20 % for 15 min.

3 Label with the date and DS (Destaining Solution). Use within kit life time.

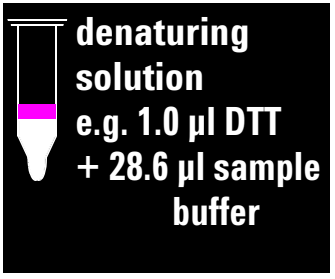
NOTE The prepared destaining solution is sufficient for 25 chips and is stable for the complete kit lifetime.

Preparing the Denaturing Solution

1 Add 3.5 Vol-% of 1 M Dithiothreitol or ß-mercaptoethanol to your aliquot of sample buffer, white ❍ (e.g. 1.0 µl DTT or BME to an aliquot of 28.6 µl Sample Buffer).

2 Vortex for 5 s.

3 For non-reducing conditions, add 3.5 Vol-% of water to your aliquoted sample buffer vial and vortex afterwards for 5 s.

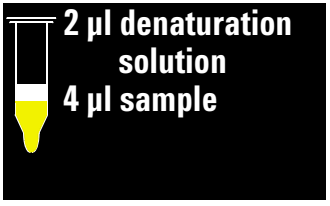


NOTE The total volume of the accordingly prepared denaturing solution (200 µl sample buffer plus 7 µl 1 M DTT or water) is sufficient for 10 chips. Use the prepared denaturing solution within 1 week.
To avoid freeze thaw cycles, store the denaturing solution as well as smaller aliquots of sample buffer at 4 °C when not in use for more 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 29.

1. Combine 4 µl protein sample and 2 µl denaturing solution in a 0.5 ml vial.
2. Place the sample vials and a vial containing a 6 µl aliquot of Protein 80 Ladder ● (yellow) in the heating block or the water bath at 95 °C for 5 min. Cool down afterwards.
3. Spin vials for 15 s.
4. Add 84 µl deionized water to samples and ladder and vortex.



NOTE The diluted samples and ladder are stable for one day.
Store samples at 4 °C when not in use for more than 1 hour.

Always make aliquotes of the reagents to maintain a constant quality! E.g. you might want to prepare twenty five 6 µl aliquots of ladder (amount needed for one chip) and store them at - 20 °C.

Loading the Gel-Dye Mix and the Destaining Solution

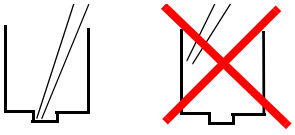
NOTE Before loading the gel-dye mix, make sure that the base-plate of the Chip Priming Station is in position (A) and the adjustable clip is set to the middle position. Refer to "Setting up the Chip Priming Station" on page 8 for details.

1 Allow the gel-dye mix to equilibrate to room temperature for 30 minutes before use. Always protect the gel-dye mix from light during this time.

2 Take a new Protein chip out of its sealed bag and put it on the Chip Priming Station.

3 Pipette 12 µl of gel-dye mix at the bottom of the well marked G.

NOTE When pipetting the gel-dye mix, make sure not to draw up particles that may sit at the bottom of the gel-dye mix vial. Insert the tip of the pipette to the bottom of the chip well when dispensing. This prevents a large air bubble forming under the gel-dye mix. Placing the pipette at the edge of the well may lead to poor results.



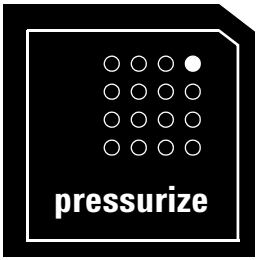
4 Set the timer to 60 seconds, make sure that the plunger is positioned at 1 ml and then close the Chip Priming Station. The lock of the latch will click when the Priming Station is closed correctly.

5 Press the plunger of the syringe down until it is held by the clip.

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

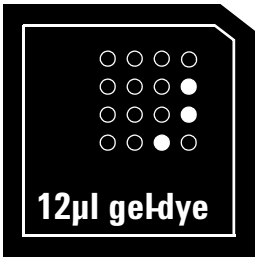
7 Visually inspect that the plunger moves back at least to the 0.3 ml mark.

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

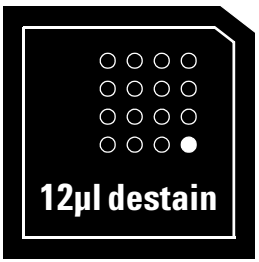


9 Open the Chip Priming Station.

10 Pipette 12 µl of gel-dye mix in all wells labelled with “G”.



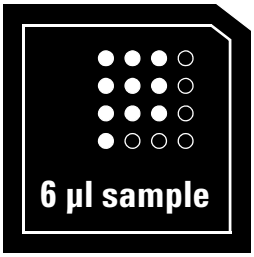
11 Pipette 12 µl of destaining solution in the well marked DS.



NOTE Protect the gel-dye mix from light. Store the gel-dye mix at 4 °C when not in use for more than 1 hour.

Loading the Ladder and Samples

1 Pipette 6 µl of sample (prepared as described in "Preparing the Samples and the Ladder" on page 15) in all 10 sample wells (Note: all 10 sample wells must be filled either with ladder or sample).



2 Pipette 6 µl of the prepared ladder into the well marked with the ladder symbol .

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

3 Place the chip in the Agilent 2100 bioanalyzer and start the assay immediately.

Inserting a Chip in the Agilent 2100 Bioanalyzer

1 Open the lid of the Agilent 2100 bioanalyzer.

2 Check that the electrode cartridge is inserted properly and the chip selector is in position (1). Refer to "Setting up the Bioanalyzer" on page 9 for details.

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

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

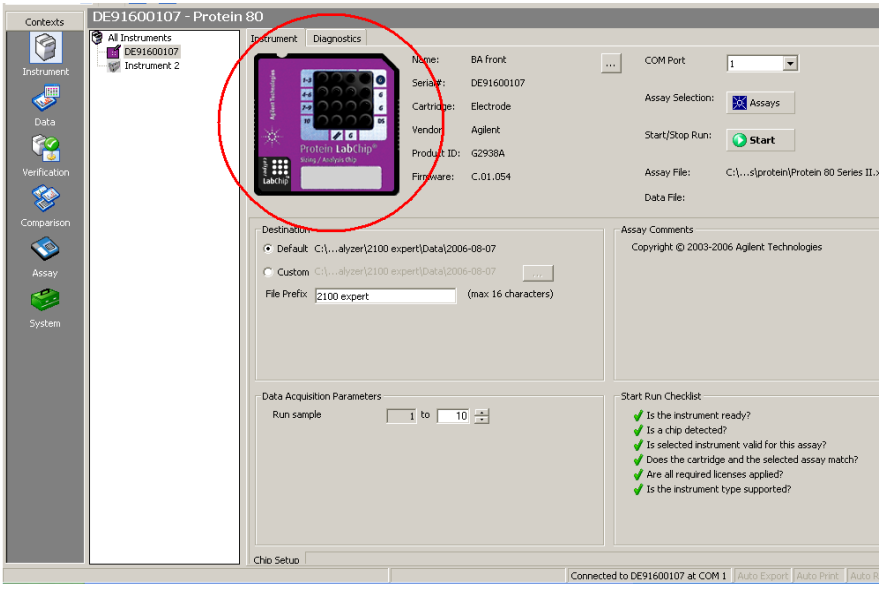
CAUTION

Sensitive electrodes and liquid spills

Forced closing of the lid may damage the electrodes and dropping the lid may cause liquid spills resulting in bad results.

⇒ Do not use force to close the lid and do not drop the lid onto the inserted chip.

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 the *Instrument* context.

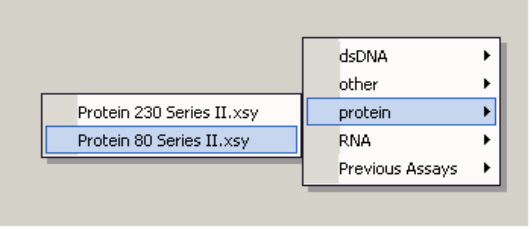


Starting the Chip Run

NOTE

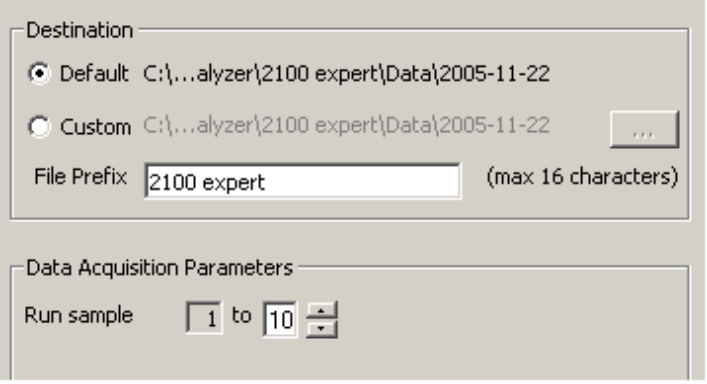
Please note that the order of executing the Chip Run may change if the Agilent Security Pack software (only applicable for Agilent 2100 expert software revision B.02.02 and higher) is installed. For more details please read the 'User's Guide' which is part of the Online Help of your 2100 expert software.

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



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

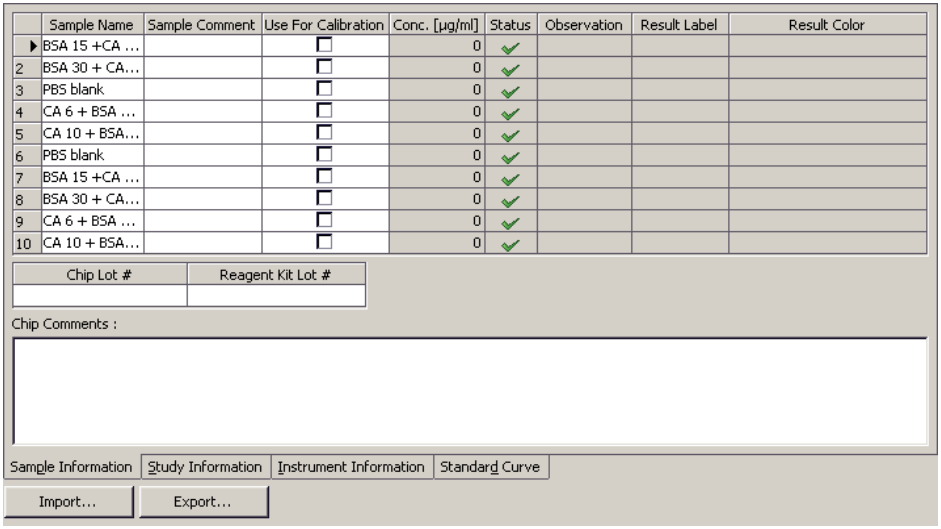
Data will be saved automatically to a file with a name using the prefix you have just entered. At this time you can also customize the file storage location and the number of samples that will be analyzed.



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* context and select the *Chip Summary* tab. Complete the sample name table.

NOTE If absolute quantitation is required with a standard protein, mark the check box *Use For Calibration* and enter standard concentration.



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

6 After the chip run is finished, remove the chip from the receptacle of the bioanalyzer and dispose it according to good laboratory practices.

CAUTION

Contamination of electrodes

Leaving the chip for a period longer than 1 hour in the bioanalyzer may cause contamination of the electrodes.

⇒ Immediately remove the chip after a run.

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).

NOTE

Use a new electrode cleaner with each new kit.

CAUTION

Leak currents between electrodes

Liquid spill might cause leak currents between the electrodes.

⇒ Never fill too much water in the electrode cleaner.

1 Slowly fill one of the wells of the electrode cleaner with 350 µl deionized analysis-grade water.

2 Open the lid and place the 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 to allow the water on the electrodes to evaporate before closing the lid.

NOTE

After 5 chip runs, empty and refill the electrode cleaner.

After 25 chip runs, replace the used electrode cleaner by a new one.

NOTE

When switching between different assays, a more thorough cleaning may be required. Refer to the Agilent 2100 Bioanalyzer Maintenance and Troubleshooting Help for details. This is part of the Online Help of the 2100 expert software.

Checking Your Agilent Protein 80 Assay Results

Protein 80 Ladder Well Results

To check the results of your run, select the Gel or Electropherogram tab in the *Data* context. The electropherogram of the ladder well window should resemble the one shown below.

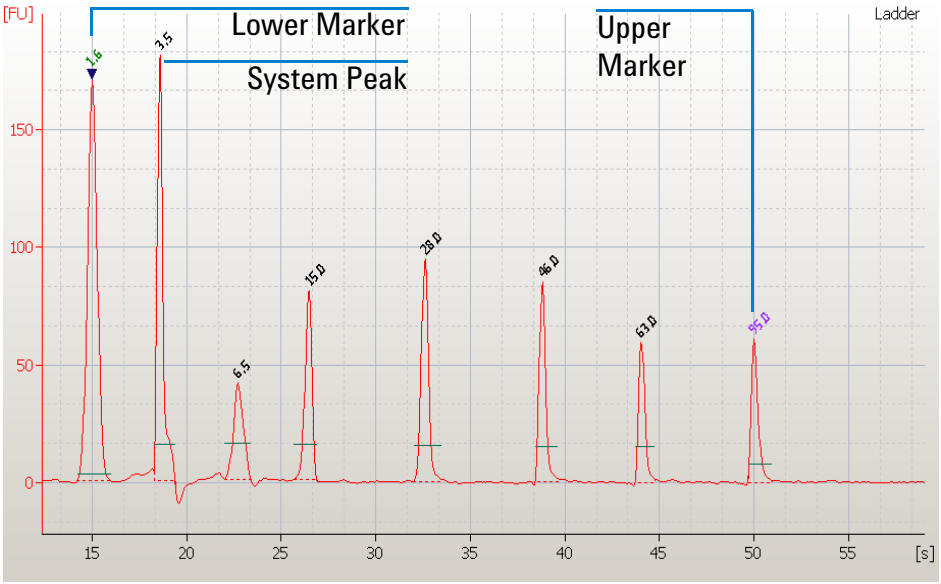


Figure 1 Protein 80 ladder

Major features of a successful ladder run are:

- 6 ladder peaks and all ladder peaks are well resolved
- Flat baseline
- Readings at least 15 fluorescence units higher than baseline readings

If the electropherogram of the ladder well window does not resemble the one shown above, for assistance, please refer to the *2100 Expert Maintenance and Troubleshooting Guide* within the online Help of the 2100 Expert software.

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.

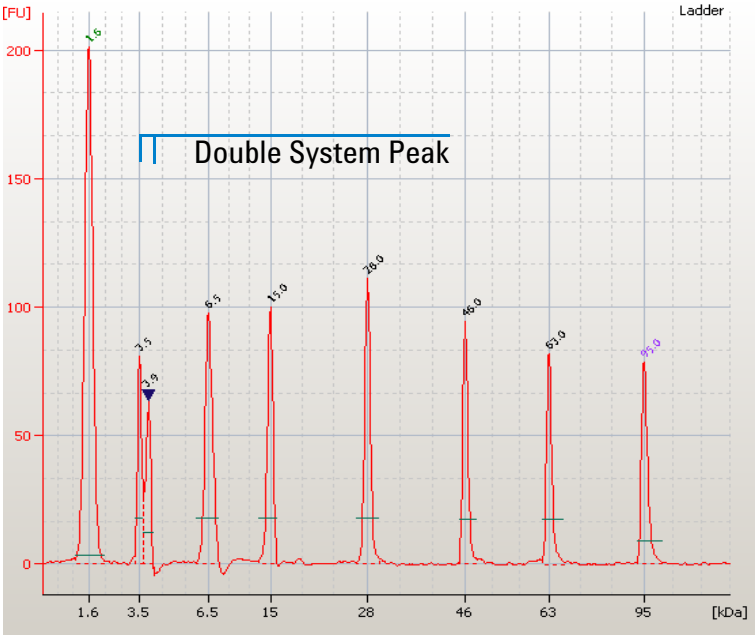
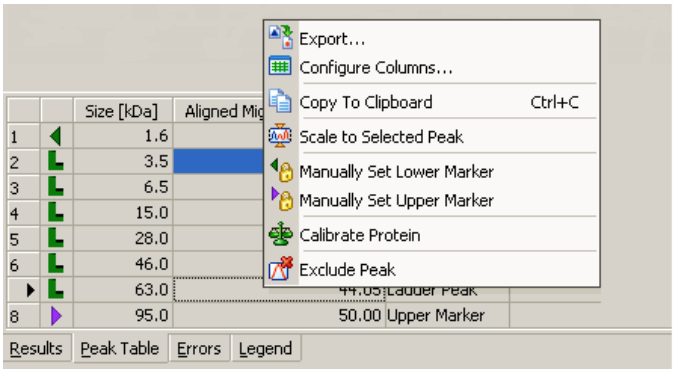


Figure 2 Protein 80 ladder with double system peak

In case both system peaks are identified as ladder peaks, exclude the first peak of the two system 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* to make the change come into effect.

Protein 80 Sample Well Results

To review the results of a specific sample, select the sample name in the tree view and highlight the *Results* sub-tab. The electropherogram of the sample well window should resemble the one shown here for the Protein 80 assay.

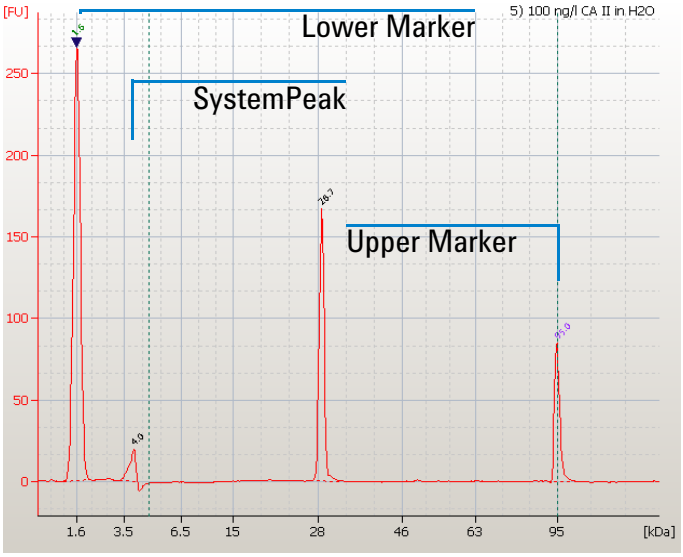


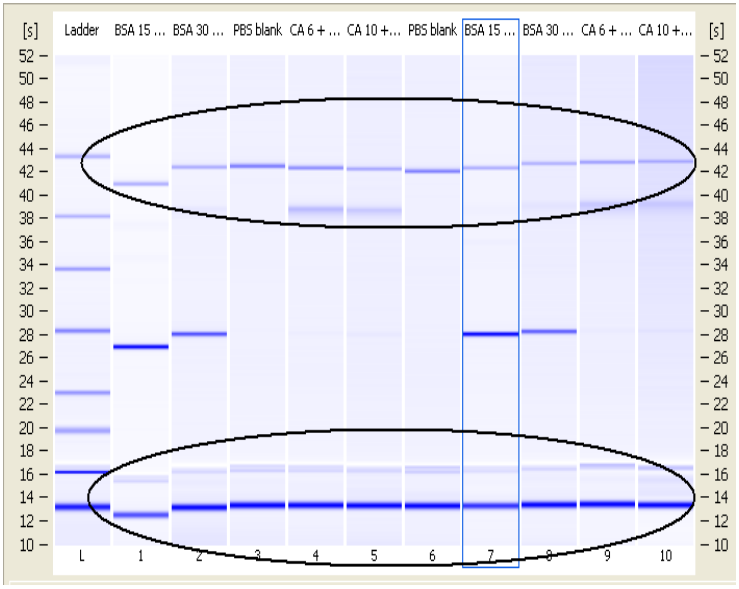
Figure 3 Protein peaks of a successful sample run

Major features of a successful protein sample run are:

- All sample peaks between the lower and upper marker peaks
- Two marker peaks, system peak(s)
- Lower marker peak between 10.5 and 15.5 seconds (raw migration time: analysis turned off by clicking the 'Don't Analyze' button)
- Upper marker peak between 38 and 48 seconds (raw migration time)
- Baseline readings between 20 and 250 fluorescence units in the ladder well (Turn analysis off)
- Both marker peaks well resolved from sample peaks (depending on sample)

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

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 *Don't analyze* and 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 80 kit.

For an updated list please refer to the web-site www.agilent.com/chem/labonachip.

Salts and Buffers (Composition Measured before Sample Preparation)
50 mM Tris / 500 mM NaCl / 500 mM imidazole pH 7.5
250 mM imidazole in PBS pH 7.4
50 mM Tris / 10 mM gluthathione pH 8.0
20 mM Tris / 100 mM NaCl / 30 mM reduced glutathion pH 7.4
50 mM $MgCl_2$ in PBS
6 M urea in PBS
25 mM HEPES / 150 mM NaCl pH 7.5
20 mM NaAc
25 mM NaF
200 mM $(NH_4)_2SO_4$
25 mM PIPES pH 7.0
100 mM Tris/150 mM sodium citrate pH 7.5
1 M NaCl (it might happen that the upper marker decreases)
PBS pH 7.4
10 mM HCl
10 mM NaOH
10 mM EDTA

Detergents	Possible Effects
1 % Triton X-100 in PBS pH 7.4	broad system peak, decreases sizing range for smaller proteins, reproducibility of quantitation might be affected, effect is less pronounced when a protein is present
0.25 % Tween 20 in PBS pH 7.4	broad system peak, decreases sizing range for smaller proteins, reproducibility of quantitation might be affected
0.375 % zwittergent E3-14 in PBS pH 7.4	large system peak, impairs sizing range
0.5 % sarcosyl in PBS pH 7.4	baseline artefact, slight hump appears around 15 kDa, less problematic if a protein is added

Other additives	Possible Effects
40 % acetonitril + 0.05 % formic acid	precipitates SDS, upper marker decreased, quantitation might be affected, maybe more spike in the baseline
1 % SDS	more diverse system peak, dip after system peak might be larger
10 % DMSO	no observations
30 % glycerol in PBS	no observations
50 mM guanidine	compatible at low concentrations, at higher concentrations than 50 mM guanidine precipitates SDS and quantitation is affected
20 % methanol + 0.25 % formic acid	precipitates SDS, upper marker decreased, quantitation might be affected
1 % PEG 2000 (polyethylene glycol)	leads to three baseline artefacts of approximately 25, 45, and 58 kDa size, reproducibility of quantitation might be affected

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In This Book

you find the procedures to analyze Protein samples with the Agilent Protein 80 Kit and the Agilent 2100 Bioanalyzer instrument.

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