

Protein 230 Kit for 2100 Bioanalyzer Systems

Kit Guide

For Research Use Only - Not for use in diagnostic procedures



Notices

Document Information

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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

1	Protein 230 Kit	4
2	Equipment Required for a Protein 230 Assay	6
3	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
4	Essential Measurement Practices	11
5	Protein 230 Assay Protocol	12
	Preparing the Gel-Dye Mix and Destaining Solution	13
	Preparing the Denaturing Solution	15
	Preparing the Samples and the Ladder	16
	Loading the Gel-Dye Mix	17
	Loading the Ladder and Samples	19
	Inserting a Chip in the 2100 Bioanalyzer Instrument	20
	Starting the Chip Run	21
	Cleaning Electrodes after a Chip Run	23
6	Checking Your Protein 230 Assay Results	24
	Protein 230 Ladder Well Results	24
	Protein 230 Sample Well Results	27
7	List of Compatible Buffers and Buffer Compounds	29



1 Protein 230 Kit

Agilent Protein 230 Kit (5067-1517)	
Protein chips	Agilent Protein 230 Reagents (5067-1518) & Supplies
25 Protein chips	● (red) Protein 230 Gel-Matrix (4 vials)
1 Electrode Cleaner	● (blue) Protein 230 Dye Concentrate (1 vial)
	○ (white) Protein 230 Sample Buffer (4 vials)
Syringe Kit	● (yellow) Protein 230 Ladder (1 vial)
1 Syringe	4 Spin Filters (5185-5990)

Table 1 Physical Specifications

Type	Specification
Analysis run time	25 min
Samples per chip	10
Sample volume	4 μ L
Kit stability	4 months
Kit size	25 chips 10 samples/chip = 250 samples/kit
Compatible buffers	"List of Compatible Buffers and Buffer Compounds" on page 29

Table 2 Analytical Specifications

Type	Agilent Protein 230 Assay
Sizing range	14 – 230 kDa
Typical sizing resolution	10 %
Typical sizing accuracy	10 % (BSA, CAII) ¹
Sizing precision	3 % CV (BSA, CAII)
Sensitivity (Signal/Noise>3)	6 ng/ μ L CAII (15 ng/ μ L BSA) in PBS 30 ng/ μ L BSA in 0.5 M NaCl
Quantitative range	15 – 2000 ng/ μ L CAII 30 – 2000 ng/ μ L BSA in PBS
Qualitative range	6 – 5000 ng/ μ L CAII 15 – 5000 ng/ μ L BSA in PBS
Quantitative precision	20 % CV (BSA, CAII)

¹ CAII = Carbonic Anhydrase, BSA = Bovine Serum Albumin, BLG = beta-Lactoglobulin

2

Equipment Required for a Protein 230 Assay

Equipment Supplied with the Agilent 2100 Bioanalyzer System

- Chip priming station (5065-4401)
- IKA vortex mixer (optional)

Additional Material Required (Not Supplied)

- Pipettes (10 μ L, 20 μ L, 100 μ L and 1000 μ L) with compatible tips
- 0.5 mL microcentrifuge tubes
- Deionized water
- 1 M Dithiothreitol (DTT) solution (recommended) or β -mercaptoethanol (BME)
- Microcentrifuge
- Heating block for 0.5 mL tubes or water bath (95 – 100 °C)

Further Information

Visit the Agilent website. It offers useful information, support, and current developments about the products and technology:

www.agilent.com/en/product/automated-electrophoresis/bioanalyzer-systems.

3

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
- finally, make sure that you start the software before you load the chip.

NOTE

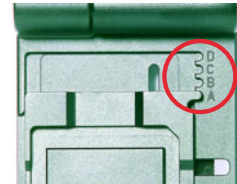
The Agilent Protein 230 assay is a high sensitivity assay. Please read this guide carefully and follow all instructions to guarantee satisfactory results.

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

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

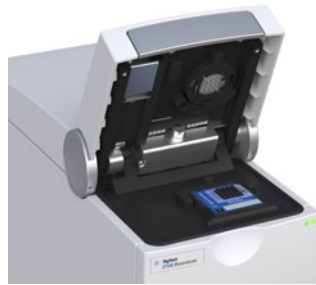


Figure 1 Electrode cartridge inserted in the instrument (graphic shows an example).

- 2 Remove any remaining chip.

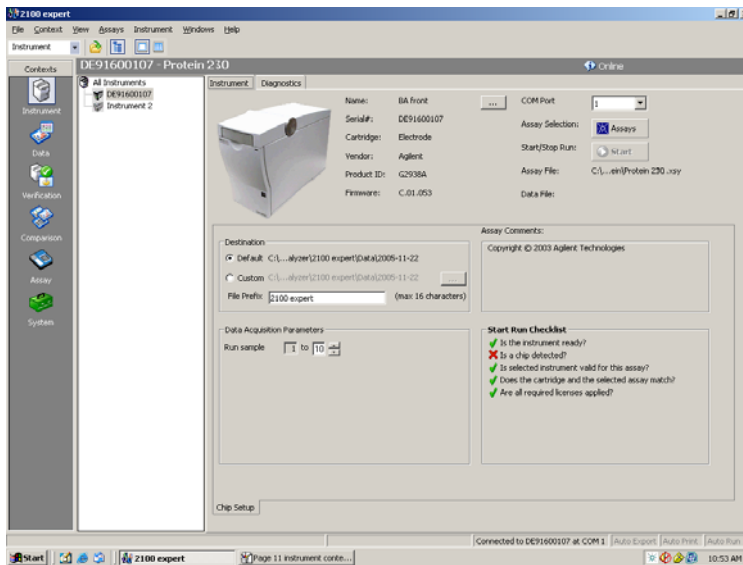
Starting the 2100 Expert Software

To start the software:

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



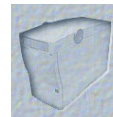
The screen of the software appears in the **Instrument** context. The icon in the upper part of the screen represents the current instrument/PC communication status:



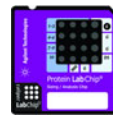
Lid closed, no chip or chip empty



Lid open



Dimmed icon: no communication



Lid closed, chip inserted,
Protein or demo assay selected

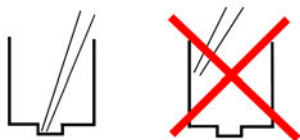
- 2 If more than one instrument is connected to your PC, select the instrument you want to use in the tree view.



4

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 required amount for a typical daily use and store them at $-28 - -15\text{ }^{\circ}\text{C}$ ($-18 - 5\text{ }^{\circ}\text{F}$). Keep the vial in use at $2 - 8\text{ }^{\circ}\text{C}$ ($36 - 46\text{ }^{\circ}\text{F}$) to avoid freeze-thaw cycles.
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes before use.
- Protect sample buffer, ladder, dye concentrate and gel-dye mix 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.



- 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 2100 Bioanalyzer instrument during analysis and never place it on a vibrating surface.
- Use 0.5 mL tubes to denature samples. Using larger tubes may lead to poor results, caused by evaporation.



5

Protein 230 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.

Preparing the Gel-Dye Mix and Destaining Solution

WARNING**Handling Reagents**

The dye and the ladder can cause serious eye irritation. Because the dye binds to nucleic acids, it should be treated as a potential mutagen.

Kit components contain DMSO. DMSO is skin-permeable and can elevate the permeability of other substances through the skin.

Dithiothreitol is harmful if swallowed and causes serious eye damage and skin irritation.

β -mercaptoethanol is fatal in contact with skin, is toxic if swallowed or inhaled, is very toxic to aquatic life with long lasting effects, causes serious eye damage, is suspected of damaging fertility or the unborn child, may cause damage to organs through prolonged or repeated exposure if swallowed, causes skin irritation, and may cause an allergic skin reaction.

- ✓ Follow the appropriate safety procedures and wear personal protective equipment including protective gloves and clothes as well as eye protection.
- ✓ Follow good laboratory practices when preparing and handling reagents and samples.
- ✓ Always use reagents with appropriate care.
- ✓ For more information, refer to the material safety data sheet (MSDS) on www.agilent.com.

-
- 1 Allow the Protein 230 dye concentrate (blue ●) and the Protein 230 gel-matrix (red ●) to equilibrate to room temperature for 30 minutes.

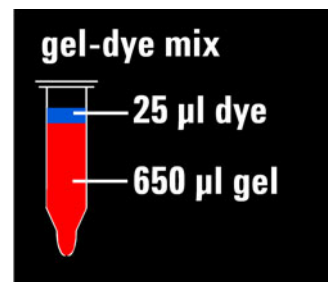
NOTE

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

Protein 230 Assay Protocol

Preparing the Gel-Dye Mix and Destaining Solution

- 2 Vortex the Protein 230 dye concentrate (blue ●) for 10 seconds and spin down. Make sure the solution is completely thawed.
- 3 Pipette 25 μ L of the dye concentrate (blue ●) into a red-capped Protein 230 gel matrix vial (red ●). Store the dye concentrate at 2 – 8 °C (36 – 46 °F) in the dark again.



NOTE

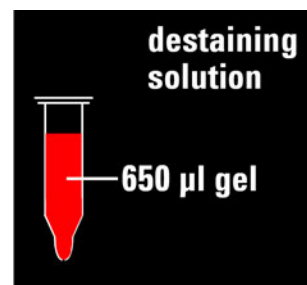
Always use the volumes indicated. Using different volumes in the same ratio will produce inaccurate results.

- 4 Cap the tube, vortex well (10 seconds at highest setting) and spin down for 15 seconds. Make sure that dye and gel are completely mixed.
- 5 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 2 – 8 °C (36 – 46 °F) when not in use for more than one hour.

- 6 For the destaining solution, transfer 650 μ L Protein 230 gel matrix (red ●) from a second vial into the top receptacle of a new spin filter and label the tube and include the date of preparation.
- 7 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 \pm 20 % (for Eppendorf microcentrifuge, this corresponds to 5200 rpm).



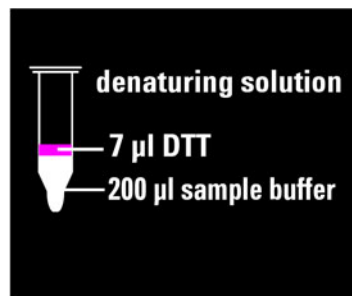
- 8 Discard the filter according to good laboratory practices.

NOTE

The prepared destaining solution is sufficient for 25 chips and is stable for the complete kit lifetime. Protect the gel-dye mix from light. Store the gel-dye mix and destaining solution at 2 – 8 °C (36 – 46 °F) when not in use for more than 1 hour.

Preparing the Denaturing Solution

- 1 Remove one of the original vials of the Protein 230 sample buffer (white ◯) or a vial with an aliquot of the Protein 230 sample buffer from the freezer. Allow to equilibrate to room temperature for 10 minutes, then vortex.
- 2 For *reducing* conditions: To the 200 μ L sample buffer in the original vial (white ◯) add 7 μ L of 1 M Dithiothreitol (DTT) solution respectively 3.5 Vol,-% of 1M DTT to your aliquot of sample buffer (e.g. 40 μ L sample buffer + 1.4 μ L DTT). Or add 7 μ L of β -mercaptoethanol (BME) to the original Protein 230 sample buffer vial.
We generally recommend to use a 1M DTT solution.



OR

For *non-reducing* conditions: Add 7 μ L of deionized water to 200 μ L sample buffer.

- 3 Vortex for 5 seconds.

NOTE

This 200 μ L denaturing solution is sufficient for 10 chips. Use the prepared denaturing solution within 2 weeks. To avoid freeze, thaw cycles, store the denaturing solution as well as smaller aliquots of sample buffer at 2 – 8 $^{\circ}$ C (36 – 46 $^{\circ}$ F) when not in use for more than 1 hour.

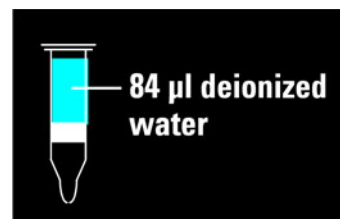
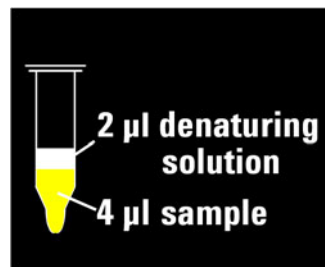
Preparing the Samples and the Ladder

NOTE

For a list of compatible buffers, please refer to the chapter “List of Compatible Buffers and Buffer Compounds” on page 29.

- 1 Allow the denaturing solution (prepared as described in “Preparing the Denaturing Solution” on page 15) and the Protein 230 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 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 more than 5 minutes otherwise excessive evaporation might occur. The samples and ladder should not dry down.
- 6 Let the tubes cool down for 10 seconds and spin them for 15 seconds.
- 7 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**

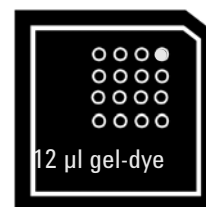
The diluted samples and ladder are stable for one day. Store samples at 2 – 8 °C (36 – 46 °F) when not in use for more than 1 hour. For your convenience you might want to prepare twenty five 6 μL aliquots of ladder (amount needed for one chip) and store them at -28 – -15 °C (-18 – 5 °F).

Loading the Gel-Dye Mix

NOTE

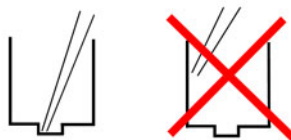
Before loading the gel-dye mix, make sure that the base plate of the chip priming station is in position (A) and the adjustable clip is set to the middle position. Refer to “Setting up the Chip Priming Station” on page 8 for details.

- 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 μ L of gel-dye mix at the bottom of the well marked **G**

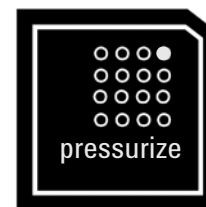


NOTE

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 s, 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 with the white **G** on black ground **G**.



Protein 230 Assay Protocol

Loading the Gel-Dye Mix

11 Pipette 12 μL of the gel- dye mix in each of the 4 wells marked **G** and **G**.



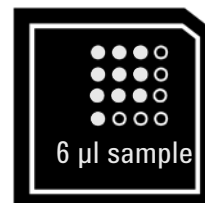
12 Pipette 12 μL of the destaining solution in the well marked **DS**.

**NOTE**

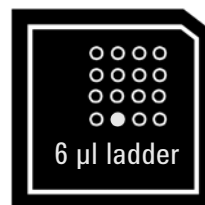
Protect the gel-dye mix from light. Store the gel-dye mix at 2 – 8 °C (36 – 46 °F) when not in use for more than 1 hour.

Loading the Ladder and Samples

- 1 Pipette 6 μL of the diluted samples (prepared as described in “Preparing the Samples and the Ladder” on page 16) into the sample wells marked 1...10.



- 2 Pipette 6 μL of the diluted 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 Make sure that the run is started within 5 minutes. Refer to the next topic on how to insert the chip in the Agilent 2100 bioanalyzer.

Inserting a Chip in the 2100 Bioanalyzer Instrument

- 1 Open the lid of the 2100 Bioanalyzer instrument.
- 2 Check that the electrode cartridge is inserted properly. 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.

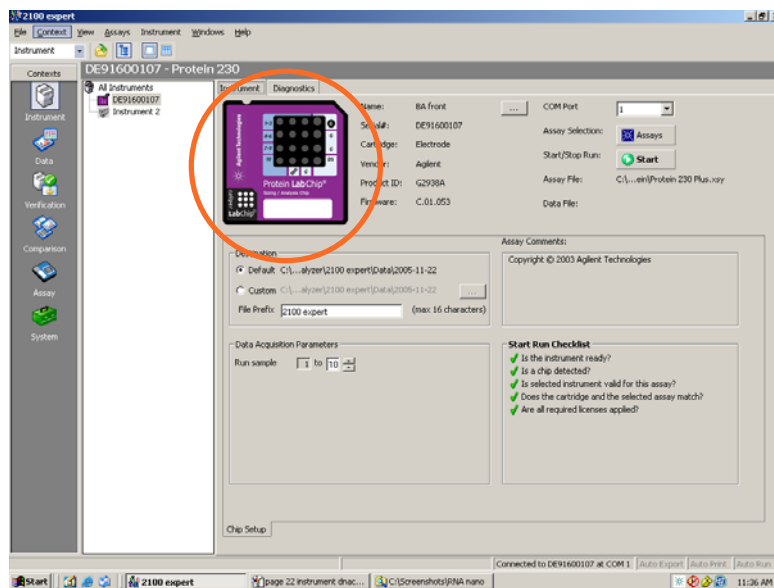
CAUTION

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.

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

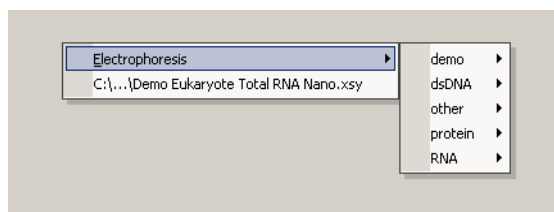


Starting the Chip Run

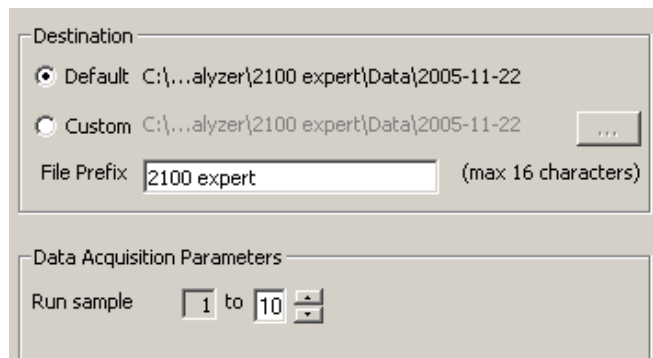
NOTE

Please note that the order of executing the chip run may change if the Agilent Security Pack software (only applicable for 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.

	Sample Name	Sample Comment	Use For Calibration	Conc. [$\mu\text{g/ml}$]	Status	Observation	Result Label	Result Color
	► Protein Ladd...		<input type="checkbox"/>	0	✓		Found Ladd...	
2	IgG reduced		<input type="checkbox"/>	0	✓			
3	Protein Ladd...		<input type="checkbox"/>	0	✓		Found Ladd...	
4	Protein Ladd...		<input type="checkbox"/>	0	✓		Found Ladd...	
5	IgG non-red...		<input type="checkbox"/>	0	✓			
6	Protein Ladd...		<input type="checkbox"/>	0	✓		Found Ladd...	
7	Protein Ladd...		<input type="checkbox"/>	0	✓		Found Ladd...	
8	IgG nonredu...		<input type="checkbox"/>	0	✓			
9	Protein Ladd...		<input type="checkbox"/>	0	✓		Found Ladd...	
10	Ladder conc.		<input type="checkbox"/>	0	✓			

Chip Lot # Reagent Kit Lot #

Chip Comments :

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



CAUTION

Contamination of electrodes

Leaving the chip for a period longer than 1 hour (e.g. over night) in the Bioanalyzer may cause contamination of the electrodes.

- ✓ Immediately remove the chip after a run.

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

Cleaning Electrodes after a Chip Run

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

NOTE

Use a new electrode cleaner with each new kit.

CAUTION

Leak currents between electrodes

Liquid spill may 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 μ L deionized analysis-grade water.
- 2 Open the lid and place the electrode cleaner in the 2100 Bioanalyzer instrument.
- 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.

6

Checking Your Protein 230 Assay Results

Protein 230 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.

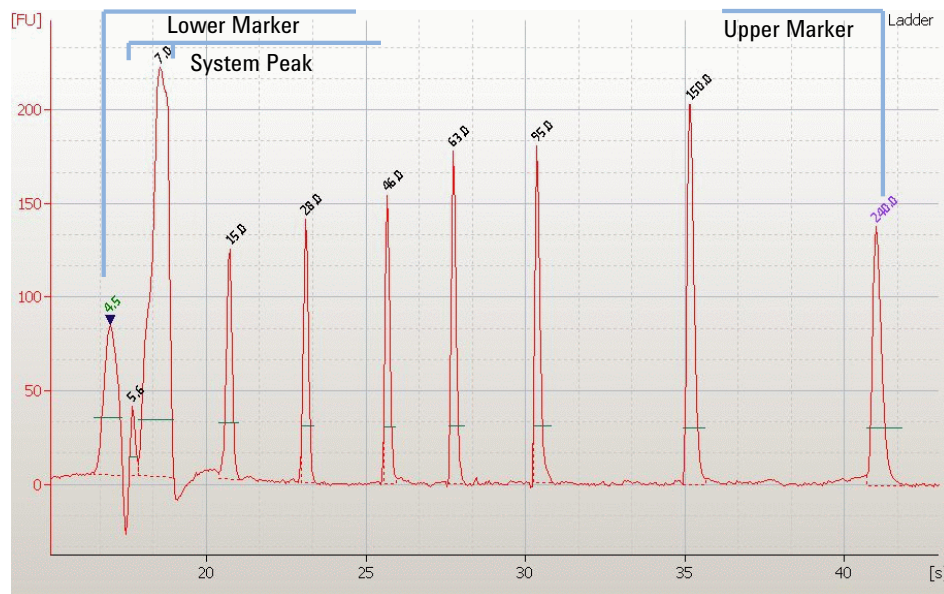


Figure 2 Protein 230 ladder

Major features of a successful ladder run are:

- 7 ladder peaks and all peaks are well resolved
- Flat baseline
- Readings at least 20 fluorescence units higher than baseline readings

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

Checking Your Protein 230 Assay Results

Protein 230 Ladder Well Results

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.

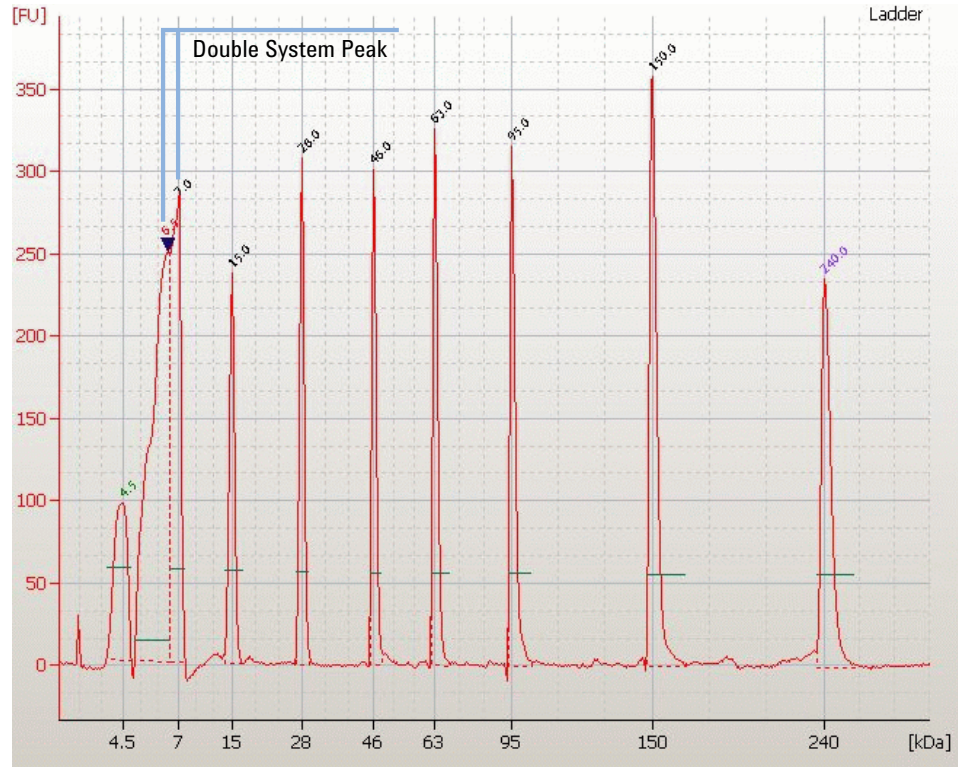


Figure 3 Protein 230 ladder with double system peak

Checking Your Protein 230 Assay Results

Protein 230 Ladder Well Results

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.

		Size [kDa]	Aligned Migration Time [s]	Time corrected area	Peak Height	Observations
1	◀	4.5	17.00	477.6	122.9	Lower Marker
2	▶	7.0	18.28	200.0	61.0	Ladder Peak
3	▶	15.0	20.65			
4	▶	28.0	23.02			
5	▶	46.0	25.59			
6	▶	63.0	27.67			
7	▶	95.0	30.33			
8	▶	150.0	35.12			
9	▶	240.0	41.00			

- Export...
- Configure Columns...
- Copy To Clipboard Ctrl+C
- Scale to Selected Peak
- Manually Set Lower Marker
- Manually Set Upper Marker
- Calibrate Protein
- Exclude Peak

- 2 Select *Exclude Peak* from ladder to make the change come into effect.

Protein 230 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 230 assay.

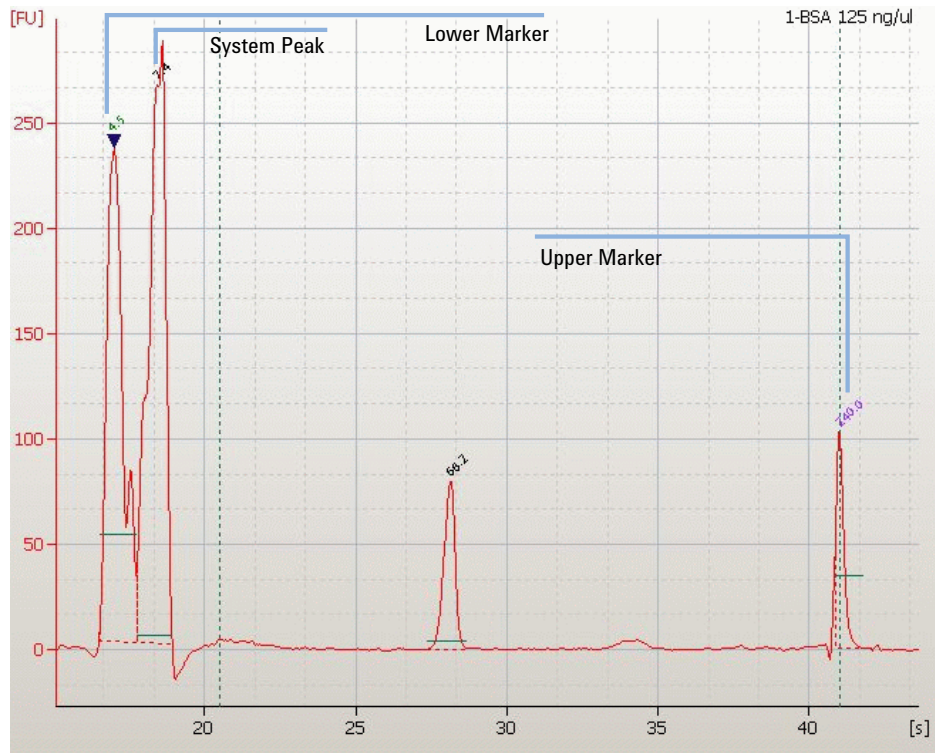


Figure 4 Protein peaks of a successful sample run

Checking Your Protein 230 Assay Results

Protein 230 Sample Well Results

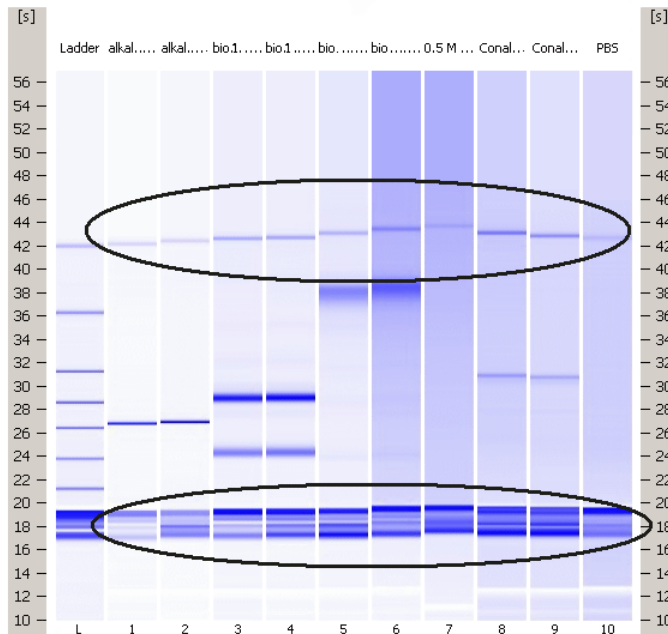
Major features of a successful protein sample run are:

- All sample peaks between the lower and upper marker peaks
- Two marker peaks, system peak(s)
- Lower marker peak between 15 and 22 seconds
- Upper marker peak between 37 and 46 seconds
- Baseline readings between 20 and 250 fluorescence units (to enable the "Don't Analyze" button, see Zero Baseline in the *2100 Expert User's Guide* or *Online Help*)
- 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 should 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*.

7

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 230 kit.

Salts and Buffers (Composition Measured before Sample Preparation)

50 mM Tris / 500 mM NaCl / 500 mM imidazole pH 7.5

20 mM Tris / 500 mM NaCl / 200 mM imidazole pH 7.9

500 mM imidazole in PBS pH 7.4

20 mM Tris / 500 mM NaCl / 200 g/ml FLAG peptide pH 7.5

50 mM Tris / 10 mM glutathione pH 8.0

20 mM Tris / 100 mM NaCl / 30 mM reduced glutathion pH 7.4

6 M urea / 50 mM NaH_2PO_4 / 100 mM NaCl / 30 mM acetic acid / 70 mM NaAc pH 5

10 mM MES / 500 mM NaAc pH 7.0

10 mM MES / 500 mM NH_4SO_4 / 10 mM NaAc pH 5.6

200 mM KCl, 40 mM MgCl_2 , 20 mM HEPES, pH 7.2

2M Urea, 15 % glycerol, 100 mM DTT, 100 mM Tris/HCl, pH 8.8

50 mM MgCl_2 in PBS

6 M urea in PBS

25 mM HEPES / 150 mM NaCl pH 7.5

20 mM NaAc

50 mM NaAc in PBS

25 mM NaF

200 mM $(\text{NH}_4)_2\text{SO}_4$

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

List of Compatible Buffers and Buffer Compounds

Protein 230 Sample Well Results

Salts and Buffers (Composition Measured before Sample Preparation)

10 mM HCl
 10 mM NaOH
 10 mM EDTA
 2.5 % mannitol
 50 mM MOPS

Detergents

Possible Effects

0.5 % CHAPS in PBS pH 7.4	large system peak, baseline hump or wave following system peak, reproducibility of quantitation might be affected, slightly affects sizing
0.25 % Triton X-100 in PBS pH 7.4	large system peak, baseline hump following system peak, reproducibility of quantitation might be affected, slightly affects sizing
0.5 % Tween 20 in PBS pH 7.4	large system peak, reproducibility of quantitation might be affected
0.25 % zwittergent E3-14 in PBS pH 7.4	large system peak
0.05 % desoxycholate in PBS pH 7.4	
0.5 % sarcosyl in PBS pH 7.4	

Other additives

Possible Effects

40 % acetonitrile 0.1 % TFA	precipitates SDS, upper marker decreased, quantitation might be affected
10 % DMSO	
30 % glycerol	
50 mM guanidine	compatible at low concentrations, at higher concentrations than 50 mM guanidine precipitates SDS and quantitation is affected
300 mM NH_4HCO_3	quantitation might be affected, slightly affects sizing
20 % methanol	precipitates SDS, upper marker decreased, quantitation might be affected
1 % PEG 2000 (polyethylene glycol)	leads to three baseline artifacts of 25, 45, and 58 kDa size, reproducibility of quantitation might be affected

In This Book

you find the procedures to analyze protein samples with the Agilent Protein 230 reagent kit and the Agilent 2100 Bioanalyzer instrument.

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