Agilent Protein 230 Kit Quick Start Guide

Protein 230 Kit (reorder number 5067-1517)	
Protein Chips	**Protein 230 Reagents (reagent reorder number 5067-1518) & Supplies**
25 Protein Chips	● (red) Protein 230 Gel-Matrix (4 vials)
1 Electrode Cleaner	● (blue) Protein 230 Dye Concentrate *
	❍(white) Protein 230 Sample Buffer (4 vials)
Syringe Kit	● (yellow) Protein 230 Ladder
1 Syringe	4 Spin Filters

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Assay Principles

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

Assay Kits

The Agilent Protein 230 kit is designed for the sizing and analysis of proteins from 14-230 kDa and can be used to analyze cell lysates, column fractions or purified proteins. The complete Protein 230 Kit Guide can be found in the online help of the 2100 expert software.

Other protein kits from Agilent:
Protein 80 kit (reorder number 5067-1515)

Storage Conditions

- Keep all reagents and reagent mixes refrigerated at 4 °C when not in use to avoid poor results caused by reagent decomposition.
- Store Protein 230 sample buffer and ladder at -20 °C upon arrival. To avoid freeze-thaw cycles, make aliquots depending on your daily use (e.g. 6 µl for ladder). The aliquot in use should be stored at 4°C.
- Protect all reagents from light. Remove light covers only when pipetting. The reagents contain dye that decomposes when exposed to light.

Agilent Technologies

Equipment Supplied with the Agilent 2100 Bioanalyzer

- Chip priming station (reorder number 5065-4401)
- IKA Vortex mixer (optional)

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 (recommended) or 2-Mercaptoethanol (BME)
- Microcentrifuge
- 0.5 ml heating block or water bath

Physical Specifications

Type	Specification
Analysis run time	25 minutes
Number of samples	10 samples/chip
Sample volume	4 µl
Kit stability	4 months (Storage Temperature see individual box)

CAII = Carbonic Anhydrase
BSA = Bovine Serum Albumin

Analytical Specifications

Type	Agilent Protein 230 Assay
Sizing range	14-230 kDa
Typical sizing resolution	10%
Typical sizing accuracy	10% CV (BSA, CAII)
Sizing reproducibility	3% CV (BSA, CAII)
Sensitivity (Signal/Noise>3)	6 ng/µl CAII (15 ng/µl BSA) in PBS 30 ng/µl (BSA) in 0.5 M NaCl
Quantitative range	15-2000 ng/µl CAII, 30-2000 ng/µl BSA in PBS
Qualitative range	6-5000 ng/µl CAII, 15-5000 ng/µl BSA in PBS
Quantitation reproducibility	20% CV (BSA, CAII)
Compatible buffers	see *List of Compatible Buffers and Buffer Compounds* in your Protein 230 Kit Guide

Setting up the Chip Priming Station

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.

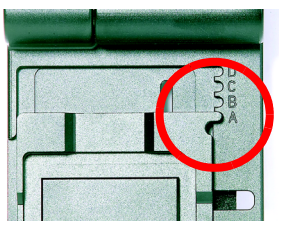
d Slide it into the hole of the luer lock adapter and screw it tightly to the priming station.

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.

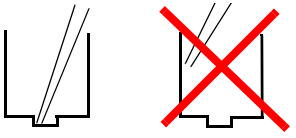


3 Adjust the syringe clip:

a Release the lever of the clip and slide it down to the middle position.

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 -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 dye, gel-dye mix, sample buffer and ladder 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 Agilent 2100 bioanalyzer 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.

Agilent Protein 230 Assay Protocol - Edition April 2007

WARNING

Handling DMSO

Kit components contain DMSO. 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 stock solutions with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

Preparing the Gel-Dye Mix

1 Add 25 µl of protein 230 dye concentrate (blue ●) to one protein 230 gel matrix (red ●) tube. Vortex well and spin down the tube for 15 s.

2 Transfer to a spin filter.

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

4 Label with the date. Use within 4 weeks.

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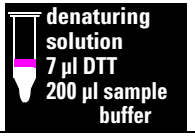
Destaining Solution

1 Pipette 650 µl of gel matrix (red ●) into a new spin filter and label the tube and include the date of preparation.
2 Centrifuge at 2500 g ± 20 % for 15 min. One tube is sufficient for 25 chips.

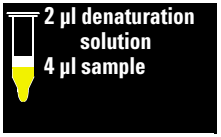
Preparing Denaturing Solution

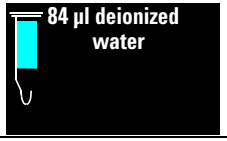
1 Add 7 µl of 1 M Dithiothreitol solution (recommended) or ß-mercaptoethanol to the sample buffer vial (200 µl, white ❍) or add 3.5 Vol-% to your aliquot of sample buffer.
2 Vortex for 5 s.



Preparing the Samples and the Ladder

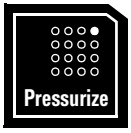
1 Combine 4 µl protein sample and 2 µl denaturing solution in a 0.5 ml tube.
2 Place sample tubes and tube with 6 µl protein 230 ladder (yellow ●) at 95-100°C for 5 min. Cool down afterwards.
3 Spin tubes for 15 s.
4 Add 84 µl deionized water to samples and ladder and vortex.





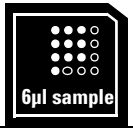
Loading the Gel-Dye Mix

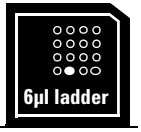
1 Adjust the base-plate of the chip priming station to position A and the syringe clip to its middle position.
2 Put a new protein chip on the chip priming station.
3 Pipette 12 µl of gel-dye mix in the well marked G.
4 Put plunger at 1 ml and close chip priming station.
5 Press plunger until held by clip, wait 60 s, then release clip.
6 Wait for 5 s. Slowly pull back plunger to 1ml position.
7 Remove solution in well G.
8 Pipette 12 µl of gel-dye mix in G and G.
9 Pipette 12 µl of destaining solution in well DS.



Loading the Ladder and the Samples

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





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

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