Protein 230 Kit for 2100 Bioanalyzer Systems

Quick Guide

The complete *Protein 230 Kit for 2100 Bioanalyzer Systems Kit Guide* can be found in the online help of the Agilent 2100 Expert software.

Kit Components

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)

For Research Use Only

Not for use in Diagnostic Procedures.

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 2100 Bioanalyzer system only.

Assay Kits

The 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 Expert software.
Other protein kits from Agilent: Protein 80 kit (5067-1515) and High Sensitivity Protein 250 kit (5067-1575)

Storage Conditions

- Keep all reagents in box labeled Part I refrigerated at 2 – 8 °C (36 – 46 °F) when not in use to avoid poor results caused by reagent decomposition.
- Store Protein 230 sample buffer and ladder (box labeled Part II) at -28 – -15 °C (-18 – 5 °F) 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 2 – 8 °C (36 – 46 °F).

- Protect all reagents from light. Remove light covers only when pipetting. The reagents contain dye that decomposes when exposed to light.
- Store the chips at room temperature.

Equipment Supplied with the Agilent 2100 Bioanalyzer System

- Chip priming station (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

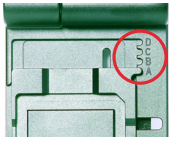
Physical Specifications		Analytical Specifications	
Analysis run time	25 min	Sizing range	14 – 230 kDa
Samples per chip	10	Typical sizing resolution	10 %
Sample volume	4 µL	Typical sizing accuracy	10 % (BSA, CAII)[1]
Kit stability	4 months	Sizing precision	3 % CV (BSA, CAII)
Kit size	25 chips 10 samples/chip = 250 samples/kit	Sensitivity (Signal/Noise>3)	6 ng/µL CAII (15 ng/µL BSA) in PBS, 30 ng/µL BSA in 0.5 M NaCl
Compatible buffers	see *List of Compatible Buffers and Buffer Compounds* in your *Protein 230 Kit for 2100 Bioanalyzer Systems Kit Guide*	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
		Quantitative precision	20 % CV (BSA, CAII)

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

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 Chip Priming Station.



2 Adjust the base-plate:

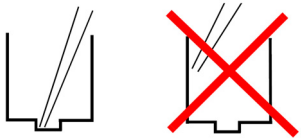
a Open the chip priming station by pulling the latch.
b Using a screwdriver, open the screw at the underside of the base plate.
c Lift the base plate and insert it again in position A. Retighten the screw.

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.

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 °C (-18 – 5 °F). Keep the vial in use at 2 – 8 °C (36 – 46 °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.



Agilent Protein 230 Assay Protocol

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.

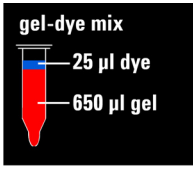
Dithiothreitol is harmful if swallowed and causes serious eye damage and skin irritation.

ß-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 swalloed, 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.**

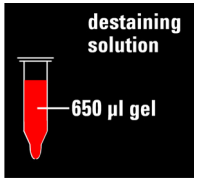
Preparing the Gel-Dye Mix

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.

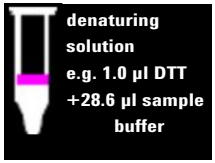


Destaining Solution

1. Transfer the content (650 µL) of another Protein 230 gel-matrix vial (red ●) to a spin filter. Make sure the complete volume of 650 µL has been transferred.
2. Centrifuge at 2500 g ± 20 % for 15 min.
3. Label with the date and DS (Destaining Solution). Use within kit life time.

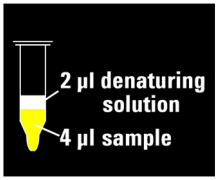


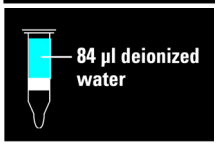
Preparing the Denaturing Solution



1 For reducing conditions, add 3.5 Vol-% of 1 M Dithiothreitol (DTT) or ß-mercaptoethanol (BME) to an aliquot of sample buffer, white ○ (e.g. 1.0 µL DTT or BME to an aliquot of 28.6 µL Sample Buffer).
OR
Alternatively, for non-reducing conditions add 3.5 Vol-% of water to your aliquoted sample buffer vial.
2 Vortex for 5 s.

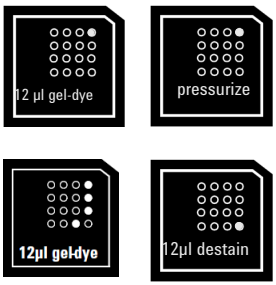
Preparing the Samples and the Ladder





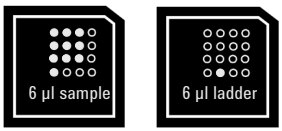
1 Combine 4 µL protein sample and 2 µL denaturing solution in a 0.5 mL tube.
2 Place sample tubes and tube with 6 µL Protein 230 ladder (yellow ●) at 95 – 100 °C for 5 min. Cool down afterwards.
3 Spin tubes for 15 s.
4 Add 84 µL deionized water to samples and ladder and vortex.

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 1 mL 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 2100 Bioanalyzer instrument and start immediately.

Technical Support

Please visit our support web page **www.agilent.com/genomics/contactus** to find information on your local Contact Center.

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**.

www.agilent.com

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