Agilent Protein 80 Kit Quick Start Guide

Protein 80 Kit (reorder number 5067-1515)	
Protein Chips	**Protein 80 Reagents (reagent 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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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 gel filled 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 80 kit is designed for the sizing and analysis of proteins from 5-80 kDa and can be used to analyze cell lysates, column fractions or purified proteins.The complete Protein 80 Kit Guide can be found in the online help of the 2100 expert software.

Other protein kits from Agilent:
Protein 230 kit (reorder number 5067-1517)

Storage Conditions

- Keep all reagents in box labelled Part I refrigerated at 4°C when not in use to avoid poor results caused by reagent decomposition.
- Store Protein 80 Sample Buffer and Ladder (box Part II) 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)

Additional Material Required (Not Supplied)

- Pipettes (10 µl, 20 µl, 100 µl, and 1000 µl) with compatible tips
- 0.5 ml microcentrifuge vials
- Deionized water
- 1 M Dithiothreitol (DTT) solution or 2-Mercaptoethanol (BME)
- Microcentrifuge
- Heating block or water bath for 0.5 ml vials

Physical Specifications

Type	Specification
Analysis run time	30 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
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
Quantitation reproducibility	20% CV (CAII, BLG)
Compatible buffers	see *List of Compatible Buffers and Buffer Compounds* in your Protein 80 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.

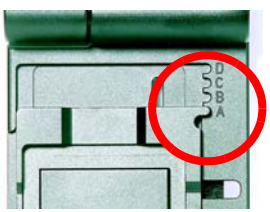
d Slide it into the hole of the luer lock adapter and screw it tightly to the chip priming station.

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.

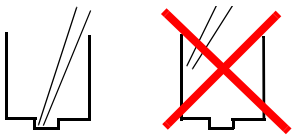


3 Adjust the syringe clip:

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 of sample buffer and 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 - Edition April 2007

WARNING

Handling DMSO

Some solutions may 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.

Preparing the Gel-Dye Mix

1 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 transfered.

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

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

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

5 Label with the date and G/D (Gel/Dye). Use within 4 weeks.

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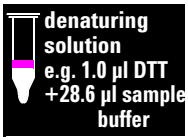
Destaining Solution

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 transferred.
2 Centrifuge at 2500 g ± 20 % for 15 min.
3 Label with the date and DS (Destaining Solution). Use within kit life time.

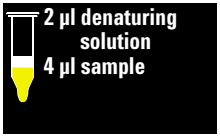
Preparing the Denaturing Solution

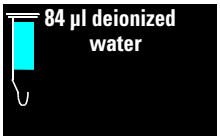
1 For reducing conditions, add 3.5 Vol-% of 1 M Dithiothreitol (DTT) or ß-mercaptoethanol (BME) to an aliquot of sample buffer (e.g. 1.0 µl DTT or BME to an aliquot of 28.6 µl Sample Buffer). Alternatively, for non-reducing conditions add 3.5 Vol-% of water to your aliquoted sample buffer vial.
2 Vortex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



Preparing the Samples and the Ladder

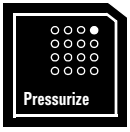
1 Combine 4 µl protein sample and 2 µl denaturing solution in a 0.5 ml vial.
2 Place sample vial and a vial containing a 6 µl aliquot of Protein 80 Ladder (● yellow) at 95 °C for 5 min. Cool down afterwards.
3 Spin tubes for 15 s.
4 Add 84 µl deionized water to samples and ladder and vortex.





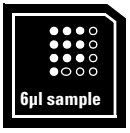
Loading the Gel-Dye Mix and the Destaining Solution

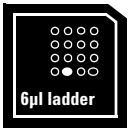
1 Adjust the base-plate of the chip priming station to position A and the syringe clip to its middle position.
2 Take a new protein chip out of the sealed bag and put it on the chip priming station.
3 Pipette 12 µl of gel-dye mix in the well marked G.
4 Put plunger at 1 ml and close chip priming station.
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 Pipette 12 µl of gel-dye mix in all wells labeled with "G".
8 Pipette 12 µl of destaining solution in well DS.



Loading the Ladder and the Samples

1 Pipette 6 µl of sample 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 in the well marked .
3 Place the chip in the Agilent 2100 bioanalyzer and start the assay 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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