Agilent High Sensitivity Protein 250 Kit Quick Start Guide

Table 1 Agilent High Sensitivity Protein 250 Kit (reorder number 5067-1575)

High Sensitivity Protein Chips	High Sensitivity Protein 250 Reagents (for separation, reorder number 5067-1576)	
10 Chips	●(red) Gel Matrix (1 vial, prefiltered)	
1 Electrode Cleaner	●(purple) Destaining Solution (1 vial)	**Syringe Kit**
	○(white) Sample Buffer (3 vials)	1 Syringe
For components used in the Agilent High Sensitivity Protein 250 labeling protocol refer to the complete kit manual.		

Assay Principles

The complete Agilent High Sensitivity Protein 250 kit contains chips and reagents for labeling of proteins with a fluorescent dye and subsequent sizing and quantitation. See the Agilent High Sensitivity Protein 250 kit guide for the required labeling procedure. This document describes the separation and detection with on-Chip- Electrophoresis. Each chip contains an interconnected set of microchannels that sieves proteins by size as they are driven through it by means of electrophoresis. The complete Agilent High Sensitivity Protein 250 Kit guide (G2938- 90310) and the individual Labeling Protocol (G2938- 90009) or this Quick Start Guide (G2938- 90008) are available through the Help-menu of the 2100 Expert Software under "related documents" or on our web- site.

Protein Kits

The Agilent High Sensitivity Protein 250 kit is designed for the sizing and sensitive analysis of proteins from 10 kDa to 250 kDa. It can be used to analyze, e.g. cell lysates, column fractions or purified proteins after an initial labeling. This kit is designed for use with the Agilent 2100 bioanalyzer only.

Other protein kits from Agilent:

- Protein 230 kit (order number 5067- 1517)
- Protein 80 kit (order number 5067- 1515)

Storage Conditions

- Keep all reagents frozen at -20 °C when not in use to avoid poor results caused by reagent decomposition.
- Protect sample buffer, destaining solution and Dye- labeled ladder/protein solution from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.
- Store chips at room temperature.

Agilent Technologies

Prerequisites for your Agilent 2100 Bioanalyzer System

- 2100 expert Software Revision B.02.06 or higher is installed
- 2100 bioanalyzer, supported are models G2938B, G2938C or G2939AA
- Chip priming station (reorder number 5065- 4401)

Additional Material Required (Not Supplied)

- 0.5 mL tubes (e.g. Protein LoBind)
- Deionized water
- Microcentrifuge
- Vortexer
- 1 M Dithiothreitol (DTT) solution
- 0.5 mL heating block or water bath

Physical Specifications		Analytical Specifications	
Type	**Specification**	**Type**	**Specification**
Analysis run time	30 min	Sizing range	10 – 250 kDa
Samples per chip	10	Typical sizing resolution	10 %
Sample volume	5 µL	Typical sizing accuracy	10 % (BSA[1])
Kit stability	6 months at -20 °C	Sizing reproducibility	3 % CV (BSA)
Kit size	10 chips 10 sample/chip = 100 samples/kit	Sensitivity (Signal/Noise > 3)	1 pg/µL (labeled BSA) in water on chip, 5 pg/µL (labeled BSA) in PBS on chip, labeling reaction at 1 ng/µL of total protein
Compatible buffers	Refer to *Agilent High Sensitivity Protein 250 Kit Guide*	Quantitative range	up to 4 orders of magnitude (0.3 – 3000 ng/µL BSA)
		Quantitation reproducibility	20 % CV (BSA)

[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:

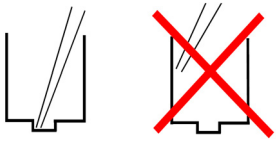
- **a** Open the chip priming station by pulling the latch.
- **b** Using a screwdriver, open the screw at the underside of the base plate.
- **c** Lift the base plate and insert it again in position A. Retighten the screw.

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

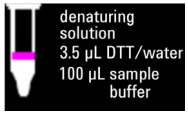
- Handle and store all reagents according to the instructions under storage conditions.
- Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.
- Allow all reagents and samples to equilibrate to room temperature for 30 min before use and vortex.
- Protect all following reagents from light: Destaining Solution, Sample Buffer, Dye-labeled ladder and Dye- labeled protein solution. Remove light covers only when pipetting. Dye decomposes when exposed to light and this reduces the signal intensity.
- Always insert the pipette tip to the bottom of the chip 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 min. 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.
- Keep suitable aliquots of the labeling reaction of the High Sensitivity Protein 250 Ladder undiluted at -20 min. Avoid freeze thaw cycles to prevent precipitation.
- The High Sensitivity Protein 250 Assay Gel- Matrix comes pre- filtered. It is ready to use after thawing.
- For protein analysis under reducing conditions a 1 M DTT solution is required.
- Samples from labeling reactions need to be diluted prior to analysis. Do not further dilute heat denatured samples.
- Relative concentrations given by software may need correction for the dilution step (e.g. 1:200).

Preparing Denaturing Solution

1 Add 3.5 µL of 1 M Dithiothreitol (DTT) solution to a sample buffer vial (100 µL, ○white), or add 3.5 Vol-% to an aliquot of sample buffer for analysis under reducing conditions. Alternatively, for non-reducing conditions add water instead of DTT.



2 Vortex for 5 s.

Dilution of labeled protein

1 For direct analysis of labeling reactions: dilute sample and ladder 1:200 in water. For alternative dilution buffers see List of Compatible Buffers in the complete kit guide. Dilution is necessary to avoid signal saturation and subsequent bias. Often, this is due to a high Lower Marker peak, representing co-migrating excess dye from the labeling reaction and Lower Marker from the sample buffer. Alternative dilution factors for sample and ladder may be applied.

2 Diluted labeled sample and ladder should be analyzed immediately. Do not use this preparation after storage.

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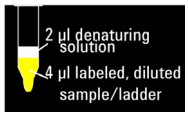
Preparing the Samples and the Ladder

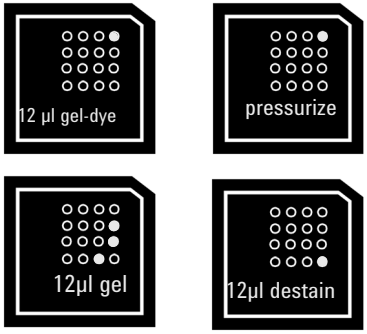


1 Combine 4 µL labeled, diluted protein sample with 2 µL denaturing solution (○ white, reducing or non-reducing) in a 0.5 mL tube.
2 Combine 4 µL of labeled, diluted High Sensitivity Protein 250 Ladder with 2 µL denaturing solution (○ white, reducing or non-reducing) in a 0.5 mL tube.
3 Place sample and ladder tubes from step 1 and 2 at 95 – 100 °C for 5 min. Cool down afterwards.
4 Spin tubes for 15 s to recover condensate of liquid.

Sample is prepared to be loaded to a chip-well. Each well per chip has to be filled, prepare duplicates of ladder or sample preparations if necessary.

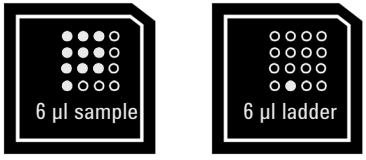
Loading the Gel

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 High Sensitivity Protein chip on the chip priming station.
3 Pipette 12 µl of gel matrix in the well marked G.
4 Put plunger at 1 mL and close chip priming station.
5 Press plunger until held by clip, wait 90 s, then release clip.
6 Wait for 5 s, then slowly pull back the plunger to the 1 mL position.
7 Open the Chip Priming Station.
8 Pipette 12 µl matrix in all remaining wells marked with G.
9 Pipette 12 µL of the destaining solution in the well marked DS.



Loading the Ladder and the Samples

- Pipette the complete volume of each denatured sample into a sample well.
- Pipette the complete volume of the denatured ladder in the well marked .
- Place the chip in the Agilent 2100 Bioanalyzer instrument and start the High Sensitivity Protein 250 Assay within 5 min.



WARNING

Handling Reagents

Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples. For kit components that are hazardous the following risk and safety phrases (Europe) apply:

- ➔ Lithium dodecyl sulfate (LiDS) in solution is Harmful (Xi), please regard R: 36/37/38 and S: 26-36.
- ➔ For Methylurea no hazard code applies, please regard R: 22 and S: 22-36.
- ➔ Fluorescent dye is Irritant (Xi), please regard: R: 41 and S: 22, 26, 39

The Material Safety Data Sheet MSDS is available under http://www.agilent.com/chem/msds

Technical Support

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

Further Information

Visit the 2100 Bioanalyzer site at http://www.agilent.com/genomics/bioanalyzer. You can find useful information, support and current developments about the products and the technology.

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