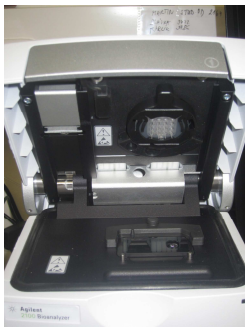
CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

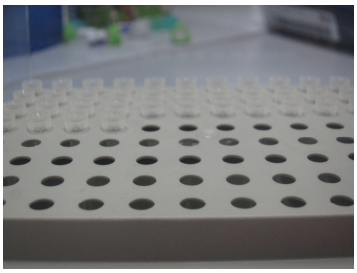
FACULTY OF TROPICAL AGRISCIENCES

Department of Crop Sciences and Agroforestry

Laboratory of Molecular Biology

Book of practicals for work with Agilent 2100 Bioanalyzer- Agilent High Sensitivity Protein Kit 250 Assay Protocol





Prague 2013

Acknowledgments

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CONTENT

1. Safety rules and regulations in Laboratory of molecular biology

- At the beginning of the work in the laboratory, students have to know laboratory safety rules and regulations in Laboratory of molecular biology.
- It is forbidden to eat, drink and smoke in the laboratory.
- Students have to wear laboratory coats, slippers and rubber gloves.
- Unauthorized experiments are strictly forbidden
- The laboratory must be kept clean and organized.
- Check the proper installation of the equipment. If there is any problem with equipment, do not use it and inform the supervisor.
- If a piece of equipment fails while being used, report it immediately to your laboratory assistant or tutor. Never try to fix the problem yourself because you could harm yourself or the others.
- Clean up your work area before leaving the laboratory.
- Turn off all electric devices before leaving the laboratory.
- Before leaving the laboratory, wash your hands.
- Ask the supervisor if you are in doubt.
- Read labels carefully.
- Never “smell” a solvent directly! Read label on the solvent bottle to identify its contents. Chemicals must never be tasted!
- Check where the laboratory fire extinguisher and wash station are located and how to use them.
- The staff and students are obliged to manipulate with poisonous, volatile and smelly substances exclusively in running hood.
- Always give the chemicals and reagents you used back to the place where you had taken them from.
- Special care should be taken while working with open fire, combustibles, corrosives and toxic substances.
- Always inform the teacher about any accident or injury and provide the first aid if necessary.
- The reagent solutions are always casted from the reagent bottle on the unlabeled side to avoid the damage of the label. Illegible inscription and incidental substitution linked with it can cause dangerous consequences.
- Concentrated acids, especially sulphuric acid, are diluted by infusion of acid into the water. Acid is infused in the thin stream to the solution which is mixed up by the glass stick throughout the whole dilution.
- Manipulation with irritating, smelling and toxic substances (i. e. chlorine, chloroform, carbon disulphide, etc.) and easily flammable substances (i. e. gasoline, acetone, etc.) is allowed only in well aired and functional hood.
- Throw toxic and nontoxic waste into the appropriate containers.
- Everybody who work in the laboratory, have to respect all the rules mentioned above and will inscribe their names into the presence book.

2. Agilent High Sensitivity Protein 250 Kit - Start Guide

Protocol for on-Chip analysis of pre-labeled proteins

Check if the Agilent High Sensitivity Protein 250 Kit content following reagements

High Sensitivity Protein Chips	**Reagents** (for separation)	
10 Chips	(red) Gel Matrix (1 vial, prefiltered)	
1 Electrode Cleaner	(purple) Destaining Solution (1 vial)	**Syringe Kit**
	(white) DNA Gel Matrix (3 vials)	1 Syringe
	3 Spin Filters	

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.

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 2lOO Bioanalyzer only.

Storage conditions

- Keep all reagents and reagents mixes refrigerated at -20°C when not in use to avoid poor results caused by reagement 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.

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

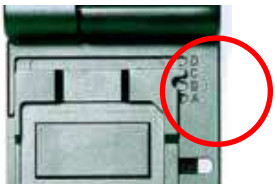
Additional Material Required

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

3. 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.

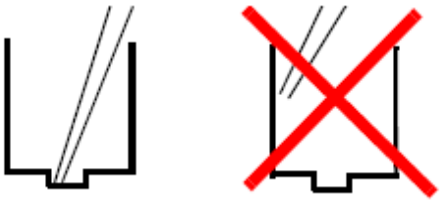
3 Adjust the syringe clip:

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

Note: **Replace the syringe with each new reagent kit.**

4. 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 minutes 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 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.
- Keep suitable aliquots of the labeling reaction of the High Sensitivity Protein 250 Ladder undiluted at -20°C. 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 lM 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).

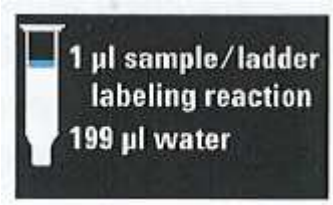
5. Agilent High Sensitivity Protein 250 Assay Protocol

Preparing Denaturing Solution

1 Add 3.5 µl of 1 M Dithiothreitol (DTT) solution to a 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

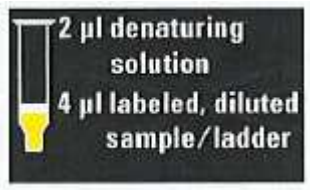


1 For direct analysis of labeling reactions: dilute sample and ladder 1:200 in water. For alternative dilution buffers see List of Compatible Buffers in the complete kit guide.
Dilution is necessary to avoid signal saturation and subsequent bias. Often, this is due to a high Lower Marker peak, representing co-migration 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.

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 labeled, diluted High Sensitivity Protein 250 Ladder with 2 µl denaturing
Solution (○ white reducing or non- reducing) in 0.5 ml tube.

3 Place sample and ladder tubes from step l and 2 at 95- 100°C for 5 min. Cool down afterwards.

4 Spin tubes for 15 s to recover condensate off 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 preparation if necessary.

Loading the Gel

1 Adjust the baseplate 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.

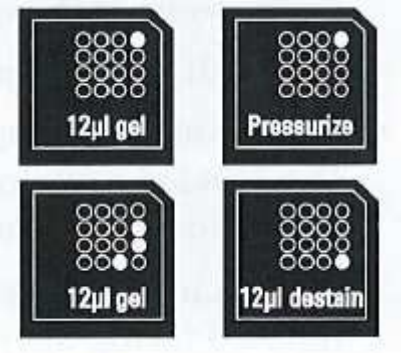
3 Pipette 12 µl of gel matrix in the well marked G.

4 Put plunger at 1 ml and close chip priming station.

5 Press plunger until held by clip, wait 90 s, then release clip.

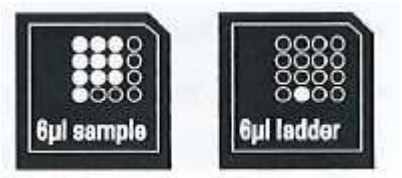
6 Wait for 5 s. Slowly pull back plunger to 1 ml position.

7 Open priming station.



8 Pipette 12 µl of gel matrix in all remaining wells marked with G.
9 Pipette 12 µl of destaining solution in well marked DS.

Loading the Ladder and the Samples



1 Pipette the complete volume of each denatured sample into a sample well.
2 Pipette the complex volume of the denaturated ladder in the well marked  .
3 Place the chip in the Agilent 2100 bioanalyzer 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 apply.
Lithium dodecyl sulfate (LiDS) in solution is harmful.
Fluorescent dye is irritant.