Agilent High Sensitivity Protein 250 Labeling Protocol

Agilent High Sensitivity Protein 250 Kit (reorder number 5067-1575)	
Agilent High Sensitivity Protein 250 Labeling Reagents (reorder number 5067-1577)	
● (green)	10x Protein 250 Standard Labeling Buffer (10xSLB), 10-fold concentrate (1 vial)
❍ (clear)	Ethanolamine (1 vial)
● (blue)	DMSO (1 vial)
● (blue)	Labeling dye (1 vial, separate light-tight bag)
High Sensitivity Protein 250 Ladder (reorder number 5067-1578)	
● (yellow)	Ladder (1 vial, sufficient for three labeling reactions)
For components used in the Agilent High Sensitivity Protein 250 on-Chip analysis 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 separation and detection with on-Chip-Electrophoresis. This document describes the labeling of proteins. The complete Agilent High Sensitivity Protein 250 Kit guide (G2938-90310) and the individual Quick Start Guide Protocol (G2938-90008) or this Labeling Protocol (G2938-90009) are available through the Help-menu of the 2100 Expert Software under "related documents" or on our web-site.

Protein Kits

The Agilent Protein 250 kit is designed for 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 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

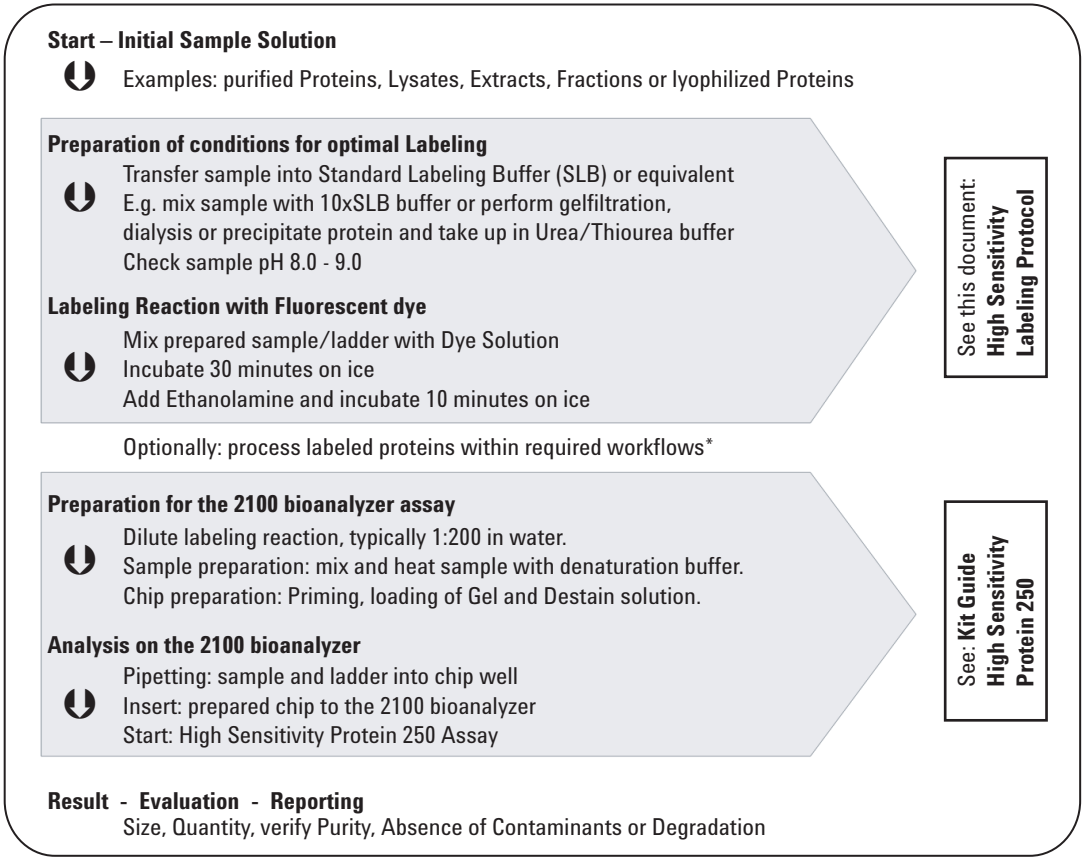
- Keep all reagents frozen at -20°C when not in use to avoid poor results caused by reagent decomposition.
- Avoid freeze thaw cycles for the ladder.
- Protect all following reagents from light: Solid and dissolved Labeling Dye and any Dye-labeled ladder/protein solution. Remove light covers only when pipetting. Dye decomposes when exposed to light.

Additional Material Required (Not Supplied)

- pH-meter or indicator strips (basic range)
- Microcentrifuge
- 0.5 ml tubes (e.g. Protein LoBind)
- Ice-bath
- De-ionized water
- Vortexer

General workflow for the Agilent High Sensitivity Protein 250 kit

For experimental details please refer to the following sections and the complete High Sensitivity Protein 250 kit guide.



*Alternative workflows may be any purification, depletion or fractionation technique (e.g. by 3100 OFFGEL fractionator, Agilent Technologies)

Sample Prerequisites	
Type	Lysates, Extracts, Column Fractions, purified Proteins, lyophilized Proteins
Concentration	1 ng/µl to 3 µg/µl total protein
Volume	5 µl per labeling reaction
pH value	Adjustment to pH 8.0 - 9.0
Optimal Matrix	Standard labeling buffer, supplied
Interferences	All components with primary amino or thiol residues

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Preparation of optimal conditions for Labeling
Please read the complete High Sensitivity Protein 250 kit guide for all relevant details.

1 pH Determination prior to the labeling reaction
Check the pH of sample solution prior to the labeling reaction. For example, transfer a droplet to a basic range pH-indicator strip or use a pH-meter. Optimal labeling with the Fluorescent Dye will take place only if the pH is between pH 8.0 and pH 9.0.

2 Verification of absence of interfering substances
Negative interference on the labeling efficiency is known from primary amine or thiol groups of buffer components such as DTT, ß-Mercaptoethanol, Glutathione, free Aminoacids and Imidazole. Detergents such as CHAPS, SDS, Triton X-100 or Tween 20 may influence the overall assay performance. Test any buffer deviating from the standard labeling buffer for suitability in the labeling reaction. Consider a transfer of the sample proteins to a recommended buffer for high labeling efficiency and good reproducibility.

3 Buffer systems suitable for the Labeling Reaction
A Standard Labeling Buffer is supplied as 10-fold concentrate (10xSLB, ●). Add one part 10xSLB to 9 parts of sample. Verify pH is shifted to pH 8 -9. Otherwise transfer sample to a recommended buffer.

Recommended Buffers	
Standard Labeling buffer	30 mM Tris/HCl, pH 8.5 (supplied as 10x concentrate, ●)
Urea/Thiourea buffer	30 mM Tris/HCl, 7 M Urea, 2 M Thiourea, pH 8.5
Sodiumbicarbonate buffer	100 mM $NaHCO_3$, pH 8-9

Optional preparation steps to remove interfering substances:
- Adjustment of the sample matrix pH by diluting into 1xSLB.
- Gelfiltration (e.g. spin columns) can be used for buffer exchange to 1xSLB, Sodiumbicarbonate or Urea/Thiourea buffer.
- Dissolving a pellet of sample proteins in standard labeling buffer. E.g. an Acetone precipitation allows removal of small interfering substances and proteins are easily dissolved into Urea/Thiourea buffer.
- Ultrafiltration is useful for sample concentration after dilution with 1xSLB, Sodiumbicarbonate or Urea/Thiourea buffer.

Labeling reaction with Fluorescent Dye

The Fluorescent Dye reagent needs reconstitution in DMSO. For the labeling reaction it is mixed with the prepared sample solution or ladder. Excess dye will be quenched after reaction by Ethanolamine. Ladder volume provided in this kit is sufficient for 3 independent labeling reactions. Each Ladder labeling reaction will be used for several chips.

Reconstitution of Fluorescent Dye in DMSO

1 Equilibrate Fluorescent Dye and DMSO (●, both vials) to room temperature. DMSO should be completely thawed.
2 Centrifuge dye vial at 10,000g for 2 min to collect solid dye particles at the bottom of the vial.
3 Visually localize the dye pellet and add 54 µl DMSO onto the pellet.
4 Vigorously vortex the Dye solution until all solid components are completely dissolved.
5 Label the vial with date.
Store Dye solution in the dark at -20°C up to 6 month.

Labeling reaction

1 Thaw reconstituted Dye solution completely and vortex prior to use.
2 Prepare 5 µl of Ladder (●) in a tube.
3 Prepare 5 µl of protein sample (ready for labeling, see page 3) per tube.
4 Place tubes on ice.
5 Add 0.5 µl of reconstituted Dye solution (●), vortex and spin down for 5 s.
6 Incubate 30 minutes on ice.
7 Add 0.5 µl of Ethanolamine (❍), vortex and spin down for 5 s.
8 Incubate 10 minutes on ice to quench any dye excess and to finalize the labeling reaction.
9 Start analysis of the labeled Ladder and store remaining solution aliquoted (1µl) at -20°C.
10 Start analysis of the labeled products or store labeling reaction mixture at -20°C.

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:

- Fluorescent dye is Irritant (Xi) please regard: R: 41 and S: 22, 26, 39.
- Ethanolamine is Corrosive (C) please regard R: 20/21/22, 34 and S: 1/2, 26, 36/37/39, 45.

Other kit components, namely DMSO, have no code for hazardous potential or associated risk and safety phrases. Please handle the DMSO stock solutions with particular caution as DMSO is known to facilitate the entry of organic molecules, such as the fluorescent dye, into tissues.

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

Technical Support Please visit our support web page **www.agilent.com/chem/contactus** to find contact information of your local Contact Center. *In Japan:* please use: email_japan@agilent.com.

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

G2938-90009

Part Number: G2938-90009
Edition 03/2008
Printed in Germany

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76337 Waldbronn, Germany