High Sensitivity Protein 250 Kit for 2100 Bioanalyzer Systems Labeling Protocol

Quick Guide

The complete *High Sensitivity Protein 250 Kit for 2100 Bioanalyzer Systems Kit Guide* can be found in the online help of the Agilent 2100 Expert software.

Kit Components

This table shows only the relevant contents of the kit. For an overview of the complete contents of the Kit 5067-1575, see the *High Sensitivity Protein 250 Kit for 2100 Bioanalyzer Systems Kit Guide*.

Agilent High Sensitivity Protein 250 Kit (5067-1575)	
High Sensitivity Protein Chips	**Agilent High Sensitivity Protein 250 Labeling Reagents (5067-1577)**
10 Chips	● (green) 10x Protein 250 Standard Labeling Buffer (10xSLB), 10-fold concentrate (1 vial)
1 Electrode Cleaner	○ (clear) Ethanolamine (1 vial)
	● (blue) DMSO (1 vial)
Syringe Kit	● (blue) Labeling Dye (1 vial, separate light-tight bag)
1 Syringe	**High Sensitivity Protein 250 Ladder (5067-1578)**
	● (yellow) Ladder (1 vial, sufficient for three labeling reactions)

For Research Use Only

Not for use in Diagnostic Procedures.

Assay Principles

The complete High Sensitivity Protein 250 kit contains chips and reagents for labeling of proteins with a fluorescent dye and subsequent sizing and quantitation. See the High Sensitivity Protein 250 kit guide for the separation and detection with on-Chip-Electrophoresis. This document describes the labeling of proteins. The complete High Sensitivity Protein 250 kit guide and the individual Quick Guide or this Labeling Protocol are available through the Help-menu of the Expert software under "related documents" or on the Agilent website **www.agilent.com**.

Protein Kits

The High Sensitivity 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 system only.
Other protein kits from Agilent: Protein 230 kit (5067-1517) and Protein 80 kit (5067-1515)

Storage Conditions

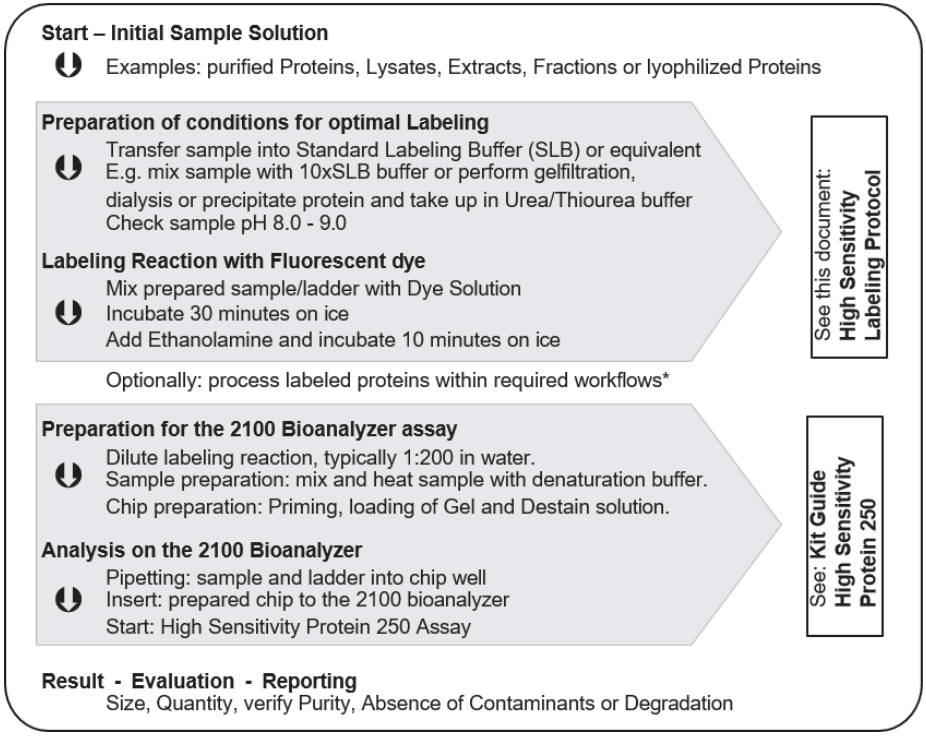
- Keep all reagents frozen at -28 – -15 °C (-18 – 5 °F) when not in use to avoid poor results caused by reagent decomposition.
- Avoid freeze thaw cycles for the ladder.
- Protect all following reagents from light: Solid and dissolved Labeling Dye and any Dye-labeled ladder/protein solution. Remove light covers only when pipetting. Dye decomposes when exposed to light.
- Store the chips at room temperature.

Additional Material Required (Not Supplied)

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

General workflow for the 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

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

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 amino acids 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 10,000 g 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 -28 – -15 °C (-18 – 5 °F) up to 6 months.

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 "Preparation of Optimal Conditions for Labeling" on 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 min on ice.
7 Add 0.5 µL of Ethanolamine (○), vortex and spin down for 5 s.
8 Incubate 10 min 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 (1 µL) at -28 – -15 °C (-18 – 5 °F).
10 Start analysis of the labeled products or store labeling reaction mixture at at -28 – -15 °C (-18 – 5 °F).

WARNING

Handling Reagents

The dye can cause serious eye damage. Because the dye binds to nucleic acids, it should be treated as a potential mutagen.

The ladder can cause eye irritation.

Kit components contain DMSO. DMSO is skin-permeable and can elevate the permeability of other substances through the skin.

Thiourea is harmful if swallowed, suspected of causing cancer and of damaging the unborn child, and is toxic to aquatic life with long lasting effects.

- ✓ **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.**

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

Printed in Germany, Edition: 11/2022

Part No: G2938-90009 Rev. D.00
Document No: SD-UF0000033 Rev. D.00

Agilent