INSTRUCTIONS

Maleimide Activated Streptavidin

21102 2064.2

Number	Description
21102	**Maleimide Activated Streptavidin,** 1mg

Storage: Upon receipt store product desiccated at 4°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific™ Maleimide Activated Streptavidin is for directly preparing streptavidin-protein conjugates with proteins, peptides and other molecules that contain a free sulfhydryl (-SH) group. Streptavidin has been maleimide-activated using Sulfo-SMCC, a heterobifunctional crosslinker that contains an *N*-hydroxysuccinimide ester and a maleimide group. The activated streptavidin presents an available maleimide group that reacts with sulfhydryl-containing molecules.

Streptavidin is a biotin-binding protein that was originally isolated from *Streptomyces avidinii* but is now produced recombinantly. Streptavidin has no carbohydrate regions, a mass of 53,000 daltons and a near-neutral pI. Streptavidin is a tetrameric protein, with each subunit binding one molecule of biotin with affinity similar to avidin. Guanidinium chloride will dissociate avidin and streptavidin into subunits, but streptavidin is more resistant to dissociation.

Important Product Information

- Reconstitute this reagent immediately before use. When in solution, the maleimide moiety might hydrolyze and become non-reactive; therefore, do not prepare stock solutions for storage. Discard any unused reconstituted reagent.
- Avoid sulfhydryl-containing components during conjugation, as these will react with the maleimide group thereby inhibiting and reducing conjugation efficiency of the intended molecule.
- Maleimides react with sulfhydryls at pH 6.5-7.5 to form stable thioether bonds. At pH values > 7.5, reactivity toward primary amines and hydrolysis of the maleimide group can occur.

Procedure for Conjugating Antibodies to Maleimide-activated Streptavidin Protein

Note: This protocol can be modified for molecules other than antibodies.

A. Choose a Method to Prepare IgG

Use one of the following strategies to ensure sulfhydryl groups are available for conjugation. Refer to the individual product instructions for detailed protocols. All product instructions are available from the website.

Note: After IgG modification, desalt the antibody with Maleimide Conjugation Buffer (100mM sodium phosphate, 5-10mM EDTA, pH 7.6, Product No. 77164)

- **Using 2-MEA to partially reduce antibodies to produce sulfhydryls:** 2-mercaptoethylamine•HCl (Product No. 20408) selectively cleaves between IgG heavy chains. The result is monovalent antibodies with sulfhydryls available for conjugation. This method preserves an intact and available antigen-binding site; however, antibody avidity is lowered as each half antibody has only one binding site.
- **Using solid-phase immobilized reductant to partially reduce antibodies to produce sulfhydryls:** The Immobilized Reductant Column (Product No. 77701) and Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) reduce disulfide bonds to produce free sulfhydryls similar to 2-MEA. Immobilized reductants enable reduction while recovering the sample in the absence of reducing agents.

- **Using SATA to add sulfhydryl groups:** SATA (Product No. 26102) is a sulfhydryl-containing modification reagent that reacts with primary amines ($-NH_2$) present on the side-chain of lysine residues. The reaction results in antibodies with protected sulfhydryl groups that can be exposed when desired. With this sulfhydryl addition method there is no risk of completely reducing and fragmenting antibodies; however, disruption of antigen-binding capability is possible from modification of antigen-binding sites, especially if binding sites contain many lysine residues.

  Note: Reagents required for the SATA method are available individually or as a complete kit (i.e., Sulfhydryl Addition Kit, Product No. 23460).

- **Using Traut's Reagent (2-iminothiolane or 2-IT) to add sulfhydryl groups:** Traut's Reagent is a cyclic thioimidate sulfhydryl-containing modification reagent that reacts with primary amines ($-NH_2$). Sulfhydryl addition with this reagent maintains the charge properties similar to the original amino group. Once added, sulfhydryl groups are available for the labeling reaction.

B. Conjugate IgG to Maleimide Activated Streptavidin

This method uses approximately three-fold molar excess of Maleimide Activated Streptavidin to IgG. For SATA- or Traut's Reagent-modified IgG, the result will be 1-3 moles of streptavidin incorporated per mole of IgG. For reduced IgG the result will be 1 mole of streptavidin incorporated per half antibody. Other molar ratios may be used.

1. Add 333µL (~2.5mg/mL) of the prepared antibody to the vial of Maleimide Activated Streptavidin.
2. Incubate reaction for 1 hour at room temperature. To increase maleimide-activated streptavidin incorporation, extend the reaction time up to 12 hours.
3. For long-term storage, remove EDTA from conjugate by dialysis or using a desalting column. Use Pierce Peroxidase Conjugate Stabilizer (Product No. 31503) or add glycerol to 50% and store at -20°C.

Additional Information

Determine Location of Reducing Reagents and Protein using the Pierce BCA Protein Assay

Because 2-MEA interferes with coupling, separation of 2-MEA from reduced IgG is critical. To determine if adequate separation was achieved, perform the BCA Protein Assay (Product No. 23225) to identify locations of 2-MEA and the protein.

1. Using a gravity-flow-desalting column, place microcentrifuge tubes in a rack and collect fractions as the solution flows through the column.
2. Prepare the BCA Working Reagent according to the instructions supplied with the kit. Pipette 200µL of Working Reagent into one microplate well for each fraction collected.
3. Add 5µL from each fraction to the wells. The 2-MEA reacts immediately producing an intense blue to purple product.

   Note: Do not use greater than 5µL of sample, as the EDTA content of the buffer will interfere with the assay.
4. After 15-30 minutes, wells containing protein will turn blue to purple. A blank (or green) well between protein-containing samples and 2-MEA indicates excellent separation.

Information Available from the Internet

Please visit our website for additional information including the following items:

- Tech Tip #43: Protein stability and storage
- Tech Tip #6: Extinction coefficients guide

Related Thermo Scientific Products

31007	**Maleimide Activated NeutrAvidin™ Protein,** 5 mg
77701	**Immobilized Reductant Column**
77712	**Immobilized TCEP Disulfide Reducing Gel**

20408	**2-Mercaptoethylamine•HCl** (2-MEA), 6 × 6mg in amber screw-cap vials
26102	**SATA** (*N*-succinimidyl S-acetylthioacetate), 50 mg
26103	**Hydroxylamine•HCl,** 25g
26101	**Traut's Reagent** (2-iminothiolane•HCl), 500mg
23460	**Sulfhydryl Addition Kit**
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs, each yielding 500mL
20673	**Dimethylformamide** (DMF), 50mL
77164	**Imject Maleimide Conjugation Buffer**, 30mL
89893	**Zeba Spin Desalting Columns,** 10mL, 5 columns, for 700-4000µL samples
23225	**BCA Protein Assay Kit**
23236	**Coomassie Plus (Bradford) Assay Kit**
31503	**Pierce Peroxidase Conjugate Stabilizer,** 25mL

Reference

Hiller, Y., *et al.* (1987). Biotin binding to avidin. Oligosaccharide side chain not required for ligand association. *Biochem J* **248:**167-71.