INSTRUCTIONS

Streptavidin Hydrazide

21120 0383.4

Number	Description
21120	**Streptavidin Hydrazide,** 2mg

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

Introduction

The Thermo Scientific Streptavidin Hydrazide enables preparation of streptavidin-glycoprotein conjugates. Hydrazide groups form hydrazone bonds upon reaction with carbonyls (aldehydes and ketones), which occur at the reducing end of polysaccharides or may be created by oxidation of carbohydrate groups in glycoproteins and other molecules. Detection of Streptavidin Hydrazide conjugates can be achieved using a biotinylated probe, such as biotin-horseradish peroxidase (Product No. 29139), biotin-alkaline phosphatase (Product No. 29339) or biotin-fluorescein (Product No. 22030).

Important Product Information

- The glycoprotein or other material to be labeled with the streptavidin hydrazide must have reactive aldehyde or ketone groups (carbonyls). These groups may exist at the reducing end of polysaccharides. To create additional carbonyls, oxidize sugar groups using either a specific oxidase, such as galactose oxidase, or 1-10mM sodium *meta*-periodate ($NaIO_4$; Product No. 20504). Oxidation with periodate is most efficient in acidic conditions (e.g., 0.1M sodium acetate, pH 5.5), although neutral buffers such as phosphate-buffered saline can be used. Avoid buffers that contain reducing agents such as 2-mercaptoethanol or dithiothreitol, which will interfere with oxidation. Additionally, avoid materials that can be oxidized, such as glycerol, which will compete with the reaction.
- Hydrazides react with carbonyls most efficiently in amine-free, near-neutral conditions (pH 6.5-7.5). If oxidation is performed in acidic conditions, buffer exchange by dialysis or gel filtration (see Related Thermo Scientific Products) into neutral buffer may be necessary to obtain efficient hydrazide conjugation. Avoid Tris or other primary amine-containing buffers during glycoprotein oxidation and the hydrazide reaction because they can react with the aldehyde groups, competing with the intended reaction to the hydrazide.
- Sodium azide at 0.01% may be used as an anti-microbial agent for long-term storage; however, avoid sodium azide when using this product with horseradish peroxidase (HRP) because sodium azide inhibits its activity.

Conjugating Streptavidin-Hydrazide to Glycoproteins

A. Additional Materials Required

- Glycoprotein or other carbohydrate-containing molecule
- Oxidation Buffer: 0.1M sodium acetate buffer, pH 5.5. (As described in the Important Product Information, phosphate-buffered saline, pH 7.2 may be used as an alternative but is not as efficient for periodate oxidization.)
- Phosphate-buffered saline (PBS; e.g., 100mM sodium phosphate, 150mM sodium chloride; pH 7.2; Product No. 28372)
- Sodium *meta*-periodate (Product No. 20504)
- Desalting column or other device to separate oxidized protein from sodium *meta*-periodate (e.g., Thermo Scientific Zeba Spin Desalting Columns, 5mL; Product No. 89892)
- Amber glass vial or other tube that will protect the reaction from light

B. Procedure for Glycoprotein Oxidation and Conjugation

1. Dissolve 0.5-10mg glycoprotein (e.g., polyclonal antibody) in 1mL of Oxidation Buffer.
2. Prepare 20mM periodate solution by dissolving 4.3mg of sodium *meta*-periodate per milliliter of Oxidation Buffer. Prepare a volume equal to the volume of Glycoprotein solution. Keep solution on ice and protect it from light.
3. Add 1mL of cold sodium *meta*-periodate solution to 1mL of the Glycoprotein solution and mix well. Allow the oxidation reaction to proceed in the dark for 30 minutes on ice or at 4°C. For more details, see instructions for Product No. 20504.
4. Dialyze samples overnight against PBS, or use a desalting column equilibrated with PBS to remove excess periodate and exchange the buffer to one that is more optimal for hydrazide reaction.
5. Add dissolved Streptavidin Hydrazide to the sample at 1- to 5-fold molar excess; mix for 2 hours at room temperature.

   Note: Optimal streptavidin-hydrazide concentration and reaction conditions depend on target protein and downstream application and must be determined empirically.

Labeling Glycoproteins on Nitrocellulose Membranes

This procedure is an example protocol for using this product. Specific applications require optimization. This protocol is adapted from published references.[1-3] The detection level is 0.5-5µg of glycoprotein when transferred from a gel or 1-3ng when using a dot blot.[2] For best results, optimize working solution concentrations for the biotinylated alkaline phosphatase and the streptavidin hydrazide.

A. Additional Materials Required

Note: Use 0.5-1.0mL per cm^2 of nitrocellulose for all solutions.

- Glycoproteins transferred onto a nitrocellulose membrane or dot blotted
- Phosphate-buffered saline (PBS; e.g., 100mM sodium phosphate, 150mM sodium chloride; pH 7.2; Product No. 28372)
- Blocking Solution: 1% lysozyme/1% BSA in PBS. Prepare separate 2% lysozyme and BSA solutions by dissolving 1g of each protein in 50mL of PBS. Incubate solutions at room temperature for 2 hours, then centrifuge at 2000 × *g* and remove the clear supernatants to clean tubes. Combine half (25mL) of each protein solution into a new tube to make the Blocking Solution. Retain the remaining separate protein solutions to make additional working solutions (see below). Store solutions at -20°C.
- Periodate Solution: Prepare 10mM solution by dissolving 2.2mg of sodium *meta*-periodate (Product No. 20504) per milliliter of PBS. Keep solution on ice and protect it from light (e.g., foil-wrapped or amber vial).
- Streptavidin Hydrazide Working Solution (WS): Dissolve Streptavidin Hydrazide to ~15µg/mL in 2% lysozyme PBS
- Biotinylated Alkaline Phosphatase Working Solution (WS): Dissolve and dilute Biotinylated Alkaline Phosphatase (Product No. 29339) to ~7.5 µg/ml in 2% BSA PBS
- Optimized Streptavidin-Enzyme Complex Solution: Add equal volumes of Biotinylated Alkaline Phosphatase WS and the Streptavidin Hydrazide WS, mix and incubate at room temperature for 30 minutes before use. This complex is not stable for long-term storage. Optimization of the streptavidin-enzyme complex solution is often required and can be conducted as per published methods.[1-3] In the standard method, 24 sets of a concentration series of fetuin are spotted onto nitrocellulose. Different ratios of the streptavidin-enzyme complex solution (i.e., biotinylated alkaline phosphatase:streptavidin hydrazide) are tested with a separate concentration series of fetuin.
- Alkaline-phosphatase substrate such as Thermo Scientific 1-Step NBT/BCIP (Product No. 34042)

B. Procedure for Labeling Glycoproteins on Nitrocellulose Membranes

1. Block the nitrocellulose membrane containing the spotted or transferred glycoprotein by incubating in Blocking Solution for 1-2 hours at room temperature with gentle agitation.
2. Discard Blocking Solution and wash membrane three times with PBS.
3. Add Periodate Solution, cover to protect from light, and incubate for 30 minutes at room temperature with agitation.
4. Discard Periodate Solution and wash membrane three times with PBS.
5. Add the optimized Streptavidin-Enzyme Complex Solution and incubate for 1 hour at room temperature.
6. Discard Complex Solution and wash membrane five times with PBS.
7. Add the alkaline-phosphatase substrate and allow color to develop. Rinse blot with water to stop the color development.

Related Thermo Scientific Products

20504	**Sodium *meta*-Periodate,** 25g
89891	**Zeba™ Spin Desalting Columns, 7K MWCO, 5mL,** 5/pkg
89893	**Zeba Spin Desalting Columns, 7K MWCO, 10mL,** 5/pkg
89833	**Lysozyme,** 5g
28372	**BupH™ Sodium Phosphate Buffer**, 40 packs
77012	**Nitrocellulose Membrane,** 0.2µm, 8 × 12cm, 25/pkg
77010	**Nitrocellulose Membrane,** 0.45µm, 8 × 12cm, 25/pkg
88018	**Nitrocellulose Membrane,** 0.45µm, 30cm × 3.5m, roll
29139	**Horseradish Peroxidase, Biotinylated,** 5mg
29339	**Alkaline Phosphatase, Biotinylated,** 1mg
34042	**1-Step™ NBT/BCIP,** 250mL

Cited References

1. Bayer, E.A., Wilchek, M. (1990). Avidin and streptavidin-containing probes. *Meth Enzymol* (M. Wilchek and E. Bayer, eds.), Academic Press, New York, **184:**174-85.
2. Bayer, E.A., *et al*. (1990). Direct labeling of blotted glycoproteins. *Meth Enzymol* (M. Wilchek and E. Bayer, eds.), Academic Press, New York, **184:**427-9.
3. Bayer, E.A., *et al*. (1987). Enzyme-based detection of glycoproteins on blot transfers using avidin-biotin technology. *Anal Biochem* **161:**123-31.

General References

O'Shannessey, D.J., *et al*. (1984). A novel procedure for labeling immunoglobulins by conjugation to oligosaccharide moieties. *Immuno Lett* **8**, 273-7.

O'Shannessey, D.J. and Wilcheck, M. (1990). Immobilization of glycoconjugates by their oligosaccharides: Use of hydrazide-derivatized matrices. *Anal Biochem* **191:**1-8.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.