INSTRUCTIONS

GlycoLink™ Coupling Catalyst

88944 2313.0

Number	Description
88944	**GlycoLink Coupling Buffer,** 100mL, 0.1M sodium acetate, 0.15M sodium chloride; pH 5.5
	Aniline, 0.2mL
	Molecular Weight: 93.13

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

Note: Aniline is highly toxic. Wear gloves and handle with extreme care.

Introduction

The Thermo Scientific GlycoLink Coupling Catalyst is an enhanced buffer composed of Thermo Scientific GlycoLink Coupling Buffer and aniline for use in aldehyde-hydrazide/alkoxyamine coupling chemistry. The generation of reactive aldehydes through sodium periodate oxidation of glycoprotein *cis*-diols, in conjuction with GlycoLink Coupling Catalyst and hydrazide/alkoxyamine functional groups, provides a valuable tool for the site-directed addition of tags such as biotin (Figure 1). The use of aniline as a nucleophilic catalyst accelerates bond formation through a Schiff base intermediate, resulting in a reduction of the amount of molar-excess hydrazide/alkoxyamine in labeling reactions and eliminating the need for DMSO to solubilize higher concentrations of the labeling reagent. Additionally, Glycolink Coupling Catalyst decreases reaction times and increases coupling efficiency, resulting in > 90% coupling in 4 hours or less.

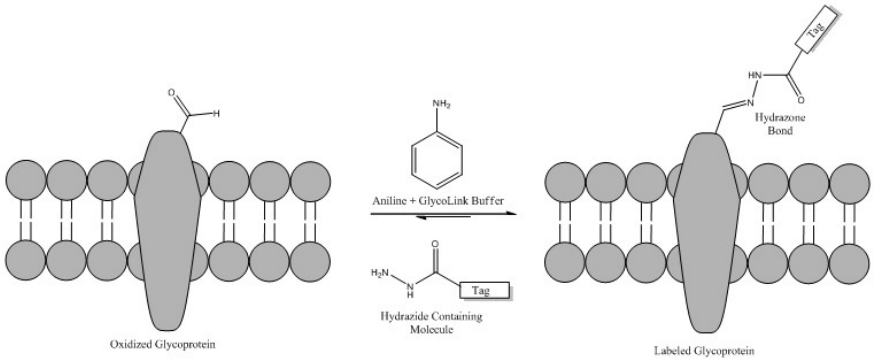

Figure 1. General reaction schematic for labeling oxidized glycoprotein using Thermo Scientific GlycoLink Coupling Catalyst and a hydrazide functional group.

Important Product Information

- **Aniline is highly toxic. Use gloves and handle with extreme care. Discard waste appropriately.**

Example Protocol for Labeling Glycoprotein with Biotin

A. Materials Required

- Hydrazide-Biotin or Alkoxyamine-Biotin (See Related Thermo Scientific Products Section)
- Sodium *meta*-Periodate (Product No. 20504)
- Purified glycoprotein

- Dialysis Cassette (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO, 0.5-3mL, Product No. 66382) or Desalting Column (e.g., Thermo Scientific Zeba Spin Desalting Columns, for desalting 0.5- 2mL samples, Product No. 89891)

B. Material Preparation

- Dissolve hydrazide-biotin or alkoxyamine-biotin to a final concentration of 5mM in GlycoLink Coupling Buffer.
- Dissolve 4.2mg of sodium *meta*-periodate in 1mL of GlycoLink Coupling Buffer. Protect from light.
- Dilute or dissolve glycoprotein to 2mg/mL in GlycoLink Coupling Buffer.

C. Procedure

1. Add 1mL of sodium *meta*-periodate solution to 1mL of glycoprotein solution; mix well while protecting reaction vessel from light. Incubate mixture for 30 minutes at room temperature.

   Note: To oxidize only sialic acid groups, add 50µL of sodium *meta*-periodate instead of 1mL (results in 1mM periodate final concentration rather than 10mM) and incubate at 4°C.
2. Remove excess periodate by dialysis against GlycoLink Coupling Buffer or gel filtration through a desalting column that has been equilibrated with GlycoLink Coupling Buffer.
3. Add 1 part prepared 5mM hydrazide-biotin solution to 9 parts oxidized and buffer-exchanged sample (results in 0.5mM hydrazide-biotin). Under a fume hood, add 9µL of aniline per milliliter of sample and mix for 1 hour at room temperature.

   Note: Aniline is highly toxic. Wear gloves and handle with extreme care.
4. Separate the biotinylated molecule from non-reacted material by dialysis or gel filtration with a desalting column.

Example Protocol for Labeling Cell Surface Glycoproteins with Biotin

A. Materials Required

- Alkoxyamine-PEG$_{(n)}$-Biotin (See Related Thermo Scientific Products Section)
- Sodium *meta*-Periodate (Product No. 20504)
- 10cm petri dish with confluent cells

B. Material Preparation

- Wash cells with 2mL of ice-cold GlycoLink Coupling Buffer. Repeat once for a total of two washes.
- Dissolve alkoxyamine-PEG$_{(n)}$-biotin to a final concentration of 1mM in GlycoLink Coupling Buffer.
- Dissolve 4.2mg of sodium *meta*-periodate in 2mL of GlycoLink Coupling Buffer (results in 10mM periodate) while protecting from light.

C. Procedure

1. Add 2mL of sodium *meta*-periodate solution to the plated cells. Incubate for 30 minutes at room temperature while protecting from light.

   Note: To oxidize only sialic acid groups, add 200µL of sodium *meta*-periodate and 1.8mL of GlycoLink Coupling Buffer (results in 1mM periodate rather than 10mM) while protecting from light.
2. Aspirate periodate solution and wash cells with 2mL of GlycoLink Coupling Buffer. Repeat wash step two times. Add 2mL of GlycoLink Coupling Buffer and 18µL of aniline to the cells.
3. Add 1 part prepared 1mM hydrazide-biotin solution to 9 parts oxidized sample (results in 0.1mM alkoxyamine-PEG$_{(n)}$-biotin). Mix for 1 hour at room temperature.
4. Wash cells in ice-cold PBS. Repeat wash step two times. Proceed to cell lysis using the method of choice.

Example Protocol for Immobilizing a Glycoprotein

Note: This procedure assumes the use of 2mL of Thermo Scientific UltraLink Hydrazide Resin and a 5mL desalting column to prepare and couple 2-20mg of purified glycoprotein in a buffer free of primary amines, carrier proteins, glycerol and antioxidants. For smaller scale immobilizations, use the GlycoLink Immobilization Kits (Product No. 88941, 88942, 88943).

A. Additional Materials Required

- UltraLink® Hydrazide Resin (Product No. 53149)
- Oxidizing Agent: Sodium *meta*-Periodate (Product No. 20504)
- 5-10mL Desalting Column (e.g., Product No. 89891, 89893)
- 5mL spin column with top and bottom caps (e.g., Product No. 29925)
- Wash Solution: 1M sodium chloride
- Thermo Scientific BupH Phosphate Buffered Saline Packs (Product No. 28372)

B. Prepare Glycoprotein Sample for Coupling (oxidize carbohydrate groups)

1. Dilute or dissolve 2-20mg of antibody or glycoprotein in GlycoLink Coupling Buffer. Dilute samples at least three-fold in GlycoLink Coupling Buffer to a final volume of 2mL and pH < 6. For example, dilute 0.6mL of 10mg/mL rabbit polyclonal IgG in 1.4mL Coupling Buffer. Alternatively, desalt to buffer-exchange into GlycoLink Coupling Buffer to a final volume of 2mL.
2. Weigh 4.2mg of sodium *meta*-periodate into an amber vial and add 2mL of glycoprotein solution, gently pipetting until the powder dissolves (results in 10mM periodate). Protect from light and incubate the mixture at room temperature for 30 minutes; to prevent over-oxidation, do not exceed the 30 minute incubation.
3. Follow the protocol to equilibrate the desalting column. Perform the desalting using the GlycoLink Coupling Buffer.
4. Slowly apply the oxidized glycoprotein solution to the center of the compact resin bed. Centrifuge and collect sample in a 15mL tube. The collected solution contains the oxidized protein. If desired, quantify protein using an aldehyde-compatible protein assay (e.g., Thermo Scientific Coomassie Plus (Bradford) Protein Assay, Product No. 23238).

C. Couple Oxidized Glycoprotein to Column Containing UltraLink Hydrazide Resin

1. Suspend the UltraLink Hydrazide Resin by end-over-end mixing. Add 4mL of resin to a 5mL spin column and centrifuge at 1000 × *g* to remove the storage buffer.
2. Add 3mL of GlycoLink Coupling Buffer and centrifuge. Repeat with an additional 3mL of GlycoLink Coupling Buffer and cap the bottom of the tube.
3. In a fume hood, add 18µL of aniline to 2mL of glycoprotein sample. Mix by pipetting up and down until aniline is completely dissolved. Save 0.1mL of the prepared sample for subsequent determination of coupling efficiency.

   Note: Aniline is highly toxic. Wear gloves and handle with extreme care.
4. Add 2mL of the oxidized glycoprotein to the GlycoLink Column.
5. Replace the top column cap and mix column by rocking or end-over-end mixing at room temperature for 2-4 hours.
6. Remove top and bottom column caps, place column into a new tube and centrifuge to collect non-bound protein.
7. Save the flow-through and determine coupling efficiency while continuing with the column wash steps. Determine coupling efficiency by comparing the protein concentrations of the non-bound fraction to the starting sample (Step F5).
8. Wash the column with 3mL of GlycoLink Coupling Buffer and centrifuge. Repeat this step two times.
9. Wash the column with 3mL of Wash Buffer and centrifuge. Repeat this step two times.
10. Equilibrate the column for storage by adding 3mL of degassed buffer at pH 7-8 (e.g., phosphate-buffered saline, PBS with optional 0.05% sodium azide) and centrifuge. Repeat this step two times.
11. Proceed to immunoaffinity purification or replace the bottom cap and add 2mL of degassed buffer at pH 7-8. Replace the top cap and store the column upright at 4°C for up to 3 weeks. Do not freeze the resin.

Troubleshooting

Problem	Cause	Solution
Low coupling efficiency	Sugars were not readily oxidizable	Increase the periodate concentration to 25mM
	Protein was not heavily glycosylated	Increase the coupling incubation time
		Choose another coupling method
	Insufficient hydrazide/alkoxyamine reagent was present	Determine the optimal concentration of hydrazide/alkoxyamine-biotin
	Primary amines and/or glycerol were not removed from the sample before coupling	Ensure primary amines and glycerol have been thoroughly removed by dialysis or desalting
	Carrier protein (BSA, gelatin) was not removed from the protein sample before coupling	Avoid BSA and gelatin in samples
		Purify the glycoprotein to remove carrier proteins

Related Thermo Scientific Products

53149 **UltraLink Hydrazide Resin,** 10mL

88941 **GlycoLink Immobilization Kit**

88942 **GlycoLink Micro Immobilization Kit**

88943 **GlycoLink IP Kit**

20504 **Sodium *meta*-Periodate,** 25g

26139 **EZ-Link Alkoxyamine-PEG$_{12}$-Biotin,** 50mg

26137 **EZ-Link Alkoxyamine-PEG$_4$-Biotin,** 50mg

26138 **EZ-Link Alkoxyamine-PEG$_4$-SS-PEG$_4$-Biotin,** 50mg

28020 **EZ-Link Hydrazide Biocytin,** 25mg

21339 **EZ-Link Hydrazide Biotin,** 100mg

21340 **EZ-Link Hydrazide-LC-Biotin,** 50mg

21360 **EZ-Link Hydrazide-PEG$_4$-Biotin,** 50mg

General References

O'Shannessy, D., *et al*. (1984). A novel procedure for labeling immunoglobulins by conjugation to oligosaccharide moieties. *Immunol Let* **8**:273-7.

Abraham, R., *et al*. (1991). The influence of periodate oxidation on monoclonal antibody avidity and immunoreactivity. *J Immunol Methods* **144**:77-86.

Dirksen, A., *et al*. (2006). Nucleophilic catalysis of hydrazone formation and transimination: Implications for dynamic covalent chemistry. *J Am Chem Soc* **128(49)**:15602-3.

Zing, Y., *et al*. (2009). High efficiency labeling of sialylated glycoproteins on living cells. *Nat Methods* **6(3)**:207-9.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce