INSTRUCTIONS

GlycoLink™ Micro Immobilization Kit

88942 2311.1

Number	Description
88942	**GlycoLink Micro Immobilization Kit,** sufficient reagents to prepare ten reusable affinity columns

Kit Contents:

GlycoLink Column, 10 × 0.1mL, UltraLink® Hydrazide Resin supplied as 50% slurry in 0.05% sodium azide

Binding Capacity: 1mg of rabbit polyclonal IgG per 0.1mL of resin

GlycoLink Coupling Buffer, 100mL, 0.1M sodium acetate, 0.15M sodium chloride; pH 5.5

Aniline, 0.2mL

Molecular Weight: 93.13

Wash Buffer, 60mL, 1M sodium chloride, 0.05% sodium azide

Sodium *meta*-periodate, 0.5g

Molecular Weight: 213.89

Zeba™ Spin Desalting Columns, 7K MWCO, 10 × 0.5mL

BupH™ Phosphate Buffered Saline Pack, 1 pack, yields 0.1M phosphate, 0.15M sodium chloride; pH 7.2 when reconstituted with 500mL of water

Column Accessories, white tips (10)

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

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

Introduction

The Thermo Scientific GlycoLink Micro Immobilization Kit contains all the components needed for immobilizing 50-500µg of glycoprotein through oxidized sugar groups. Polyclonal antibodies with abundant carbohydrates on their Fc portions and monoclonal antibodies with adequate carbohydrates are ideal for GlycoLink Chemistry, resulting in immobilizations with unobstructed antigen-binding sites and optimal purification capability. Once the desired molecule is immobilized, the resulting affinity column can be used for studying a variety of protein-protein interactions. When coupled to stable glycoproteins, columns may be regenerated and reused a minimum of five times without significant loss in binding capacity.

Immobilization is achieved using a derivatized bis-acrylamide and azlactone support in a spin-column format, enabling efficient immobilizations and purifications. The immobilization chemistry uses sodium *meta*-periodate to oxidize glycoproteins and convert sugar *cis*-diol groups to reactive aldehyde groups. The aldehydes react with hydrazide groups on the GlycoLink Column to form stable hydrazone bonds (Figure 1). Aniline is added to catalyze the reaction, resulting in > 90% coupling in 4 hours or less. Binding efficiency of the resin is dependent on the amount and type of glycosylation (Table 1).

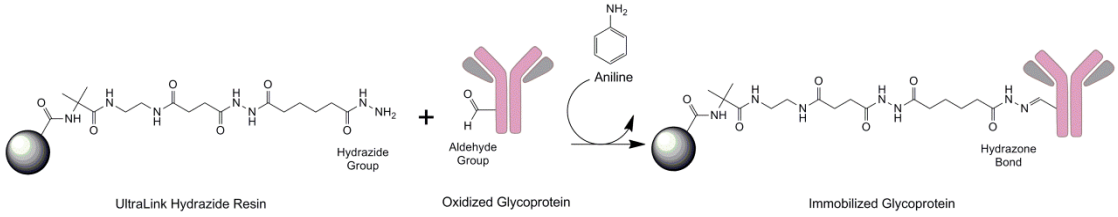

Figure 1. General structure and reaction scheme for the Thermo Scientific GlycoLink Column.

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Table 1. Coupling efficiency of glycoproteins using a 0.1mL Thermo Scientific GlycoLink Column. For each experiment, 0.4mg of protein was reacted with 0.1mL of Thermo Scientific UltraLink Hydrazide Resin using the standard protocol.

Protein	Molecular Weight	Glycosylation Sites	% Occupancy of Sites	% Coupled
Monoclonal Rat IgG_1	150,000	2	variable	72.93
Monoclonal Mouse IgG_1	150,000	2	variable	84.74
Rabbit Serum IgG	150,000	2	100	89.78
Polyclonal Chicken IgY	170,000	4	100	90.62
Human Serum IgG	150,000	2	100	97.13
Human Serum IgM	970,000	10	100	97.18
Ovalbumin	45,000	1	100	98.50

Important Product Information

- Equilibrate kit components to room temperature before processing.
- To quantify the protein and estimate coupling efficiency, use a spectrophotometer or other method.
- Primary amines (e.g., Tris, glycine), glycerol and reducing agents in the antibody solution will interfere with coupling and oxidation. Remove these components before coupling using Thermo Scientific Zeba Spin Desalting Columns or Thermo Scientific Slide-A-Lyzer Dialysis Cassettes.
- Gelatin or carrier proteins in the antibody solution will compete for coupling sites on the resin. Remove gelatin and carrier proteins using the Thermo Scientific Pierce Antibody Clean-up Kit (Product No. 44600) or by performing Protein A/G purification (Product No. 20423) and dialysis.
- Hydrophobic proteins may require additional wash steps or addition of detergent during coupling to minimize nonspecific binding.
- Particulate material in samples can impede column flow. Remove particulate by centrifugation at 10,000 × *g* or filtration using a 0.45µm membrane before applying the sample to the column.
- Perform the oxidation step in the dark. Proteins that are sensitive to oxidation (e.g., metal-containing proteins) may require shorter or milder oxidation conditions to avoid functional damage. Treatment with 1mM sodium *meta*-periodate specifically oxidizes sialic acid residues, leaving other monosaccharides unmodified. Treatment with 10-25mM sodium *meta*-periodate oxidizes other carbohydrates such as galactose or mannose.
- **Aniline is highly toxic. Wear gloves and handle with extreme care.**

Procedure for Immobilizing a Glycoprotein

Note: Perform all Thermo Scientific Zeba Spin Desalting Column and GlycoLink Column centrifugations at 1000 × *g* for 1 minute using a 2mL collection tube, unless otherwise noted. Perform all steps at room temperature and do not allow the column resin to become dry at any time.

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

1. Dilute or dissolve 50-500µg of antibody or glycoprotein in GlycoLink Coupling Buffer. Dilute samples at least three-fold in GlycoLink Coupling Buffer to a final volume of 100µL and pH < 6. For example, dilute 30µL of 10mg/mL rabbit polyclonal IgG in 70µL of GlycoLink Coupling Buffer. Alternatively, desalt to buffer exchange into GlycoLink Coupling Buffer to a final volume of 100µL.
2. Prepare 0.1M sodium *meta*-periodate by weighing 5mg into an amber vial. Dissolve with 234µL of GlycoLink Coupling Buffer. Add 11µL of stock 0.1M periodate to 100µL of glycoprotein solution. Cover tube with aluminum foil to protect from light and incubate for 30 minutes at room temperature. To prevent over-oxidation, do not exceed the 30 minute incubation. Stock periodate solution is stable for up to 1 week at 4°C when protected from light.
3. Remove the top cap and bottom tab of the 0.5mL desalting column and centrifuge.

4. To equilibrate the desalting column, add 300µL of GlycoLink Coupling Buffer and centrifuge. Repeat this step two times.
5. Slowly apply the oxidized glycoprotein solution to the center of the compact resin bed. Centrifuge and collect sample in a 1.7mL 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).
6. In a fume hood, prepare 0.2M GlycoLink Coupling Catalyst by adding 1.8µL of aniline to 100µL of GlycoLink Coupling Buffer. Vortex GlycoLink Coupling Catalyst for 10 seconds and add the total volume to the oxidized glycoprotein sample, resulting in 0.1M aniline final concentration. Continue with coupling. Save 10µL of the prepared sample for subsequent determination of coupling efficiency.

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

B. Couple Oxidized Glycoprotein to GlycoLink Column

1. Suspend the resin in the GlycoLink Column by end-over-end mixing. To avoid drawing air into the column, sequentially remove the top cap and then the bottom tab. Centrifuge the column to remove the storage buffer.
2. Add 200µL of GlycoLink Coupling Buffer and centrifuge. Repeat this step one time. Replace the bottom cap.
3. Add the oxidized glycoprotein sample to the GlycoLink Column.
4. Replace the top cap and mix column by rocking or end-over-end mixing at room temperature for 4 hours.

   Optional: Glycoproteins with greater than 3% glycosylation by weight may be incubated for < 2 hours.
5. Remove top and bottom column caps, place column into a new tube and centrifuge to collect non-bound protein.
6. Save the flow-through and determine the coupling efficiency while continuing with the column washing steps. Determine coupling efficiency by comparing the protein concentrations of the non-bound fraction to the starting sample (Step A6).
7. Wash the column with 200µL of GlycoLink Coupling Buffer and centrifuge. Repeat this step for a total of three washes.
8. Wash the column with 200µL of Wash Buffer and centrifuge. Repeat this step for a total of three washes.
9. Equilibrate the column for storage by adding 200µL of degassed buffer at pH 7-8 (e.g., phosphate-buffered saline, PBS with optional 0.05% sodium azide) and centrifuge. Repeat this step for a total of three washes.
10. Replace the bottom cap and add 200µL of degassed buffer at pH 7-8. Replace the top cap and store the column upright at 4°C. Do not freeze the resin.

General Protocol for Affinity Purification

Note: The following protocol details a spin-purification method. The amount of protein sample processed and the required binding conditions depend on the specific affinity interaction used and must be optimized for the particular experiment.

A. Materials Required

- Binding/Wash Buffer: Use phosphate-buffered saline (PBS; Product No. 29372), Tris-buffered saline (TBS; Product No. 28379) or other buffer that is conducive to forming the intended affinity interaction. Degas buffers to avoid introducing bubbles into the resin bed that may impede flow.
- Sample: Dissolve or exchange sample into Binding/Wash Buffer
- Elution Buffer: IgG Elution Buffer (Product No. 21004) or 0.1-0.2M glycine•HCl at pH 2.5-3.0
- Neutralization Buffer (optional): Prepare 1mL of 1M sodium phosphate or 1M Tris•HCl at pH 8.5-9.0

B. Method

Note: Perform all centrifugations at 1000 × *g* for 2 minutes using a 2mL collection tube.

1. Equilibrate the prepared affinity column to room temperature.
2. Remove top and bottom column caps. Centrifuge column to remove storage solution. Equilibrate column with 300µL of Binding/Wash Buffer and centrifuge.

3. Add sample (200-300µL) in appropriate binding buffer to the column. Allow sample to enter the resin bed and replace bottom cap. Add 0.2mL of Binding/Wash Buffer. Replace top cap and incubate column at room temperature while rocking or end-over-end mixing to allow binding to occur (e.g., 15-60 minutes). Alternatively, incubate column overnight at 4°C.
4. Remove top and bottom caps and centrifuge column. Save the entire flow-through to evaluate binding efficiency and capacity.
5. To wash the resin, add 200µL of Binding/Wash Buffer and centrifuge. Repeat this step 2-4 times.
6. Elute the protein with 200µL of Elution Buffer and collect into a centrifuge tube containing 10µL of Neutralization Buffer and centrifuge. Save eluted, neutralized sample and repeat this step 2-3 times.
7. Use the protein directly for SDS-PAGE or analyze by protein assay. If required for the specific downstream assay or storage, perform a buffer exchange by dialysis or gel filtration.

   Note: Equilibrate the column soon after use to prevent damage to the immobilized protein by the low-pH Elution Buffer.
8. To equilibrate the column, add 300µL of Binding/Wash Buffer to the column and centrifuge.
9. Cap the bottom and add 200µL of Binding/Wash Buffer containing a final concentration of 0.05% sodium azide for long-term storage. Cap the top and store column upright at 4°C. Do not freeze the resin.

Troubleshooting

Problem	Cause	Solution
Low coupling efficiency	Protein was not heavily glycosylated	Choose another coupling method
	Sugars were not oxidizable	Ensure sample does not contain antioxidant
	Primary amines were not removed from the sample before coupling and competed for binding to the protein	Ensure primary amines have been thoroughly removed by dialysis or desalting
	BSA, glycerol or gelatin were not removed from the protein sample before coupling and competed for binding to the column	Avoid BSA and gelatin in protein sample (See the Additional Information Section)
		Remove glycerol by desalting
Poor affinity capture	Protein was damaged during the oxidation	Shorten the oxidation time or oxidize at 4°C
Affinity column has reduced binding capacity after several uses	Immobilized protein was damaged by time, temperature or elution conditions	Prepare a new affinity column
	Binding sites and resin pores had become blocked with particulate or nonspecifically bound material	Remove precipitate from sample before affinity purification by centrifugation at 10,000 × *g* or filtration using a 0.45µm membrane
		Use nonionic detergent, high-salt concentration or other additives to reduce nonspecific binding
		Wash with greater stringency before elution

Additional Information Available on Our Website

- Tech Tip #27: Optimize elution conditions for immunoaffinity purification
- Tech Tip #29: Degas buffers for use in affinity and gel filtration columns
- Tech Tip #55: Remove BSA and gelatin from antibody solutions using Melon Gel

Related Thermo Scientific Products

89882	**Zeba Spin Desalting Columns, 7K MWCO, 0.5mL,** 25/pkg
53149	**UltraLink Hydrazide Resin,** 10mL
88941	**GlycoLink Immobilization Kit**
88943	**GlycoLink IP Kit**
88944	**GlycoLink Coupling Catalyst,** 100mL
20504	**Sodium *meta*-Periodate,** 25g
21004	**IgG Elution Buffer,** 1L
23260	**Glycoprotein Carbohydrate Estimation Kit**

General References

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.

Domen, P., *et al*. (1990). Site directed immobilization of proteins. *J Chromatogr* **510**: 293-302.

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

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