

GlycoLink™ Immobilization Kit

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Description
GlycoLink Immobilization Kit, sufficient reagents to prepare 10 reusable affinity columns

Kit Contents:
GlycoLink Column, 10 × 1mL, UltraLink® Hydrazide Resin supplied as 50% slurry in 0.05% sodium azide

Binding Capacity: 10mg of rabbit polyclonal IgG per 1mL of resin

GlycoLink Coupling Buffer, 2 × 100mL, 0.1M sodium acetate, 0.15M sodium chloride; pH 5.5

Aniline, 0.2mL

Molecular Weight: 93.13

Wash Buffer, 120mL, 1M sodium chloride, 0.05% sodium azide as a preservative

Sodium meta-periodate, 0.5g

Molecular Weight: 213.89

Zeba™ Spin Desalting Columns, 7K MWCO, 10 × 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) and column extender

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 Immobilization Kit contains all the components needed for immobilizing glycoproteins 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).

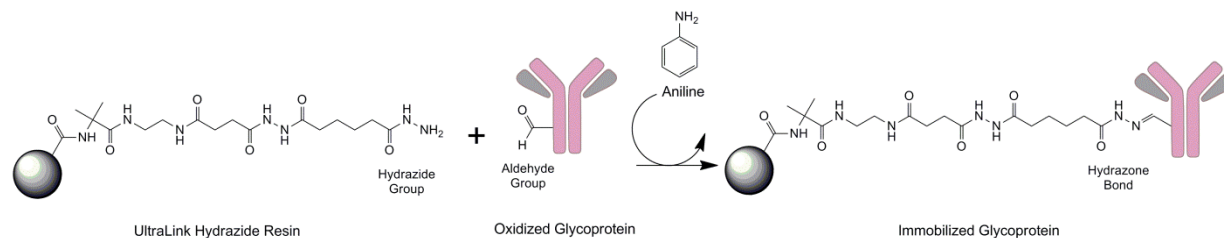


Figure 1. General structure and reaction scheme for the Thermo Scientific GlycoLink Column.

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 2 minutes at 4°C using a 15mL collection tube. Perform all other 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 1-10mg of antibody or glycoprotein in GlycoLink Coupling Buffer. Dilute samples at least three-fold in GlycoLink Coupling Buffer to a final volume of 1mL and pH < 6. For example, dilute 0.3mL of 10mg/mL rabbit polyclonal IgG in 0.7mL of GlycoLink Coupling Buffer. Alternatively, desalt to buffer-exchange the sample into GlycoLink Coupling Buffer to a final volume of 1mL.
2. Weigh 2.1mg of sodium *meta*-periodate into a 1.7mL microcentrifuge tube and add 1mL of glycoprotein solution, gently pipetting until the powder dissolves (results in 10mM periodate). Wrap tube in aluminum foil to 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. Remove the top cap and bottom tab of the 5mL desalting column and centrifuge.
4. To equilibrate the desalting column, add 2mL 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 15mL tube. The collected solution contains the oxidized protein. If desired, quantify protein by 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 18 μ L of aniline to 1mL of GlycoLink Coupling Buffer. Vortex GlycoLink Coupling Catalyst for 10 seconds and add total volume to the oxidized glycoprotein sample, resulting in 0.1M aniline final concentration. Continue with coupling and 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.

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 2mL of GlycoLink Coupling Buffer and centrifuge. Repeat this step once. 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 2mL of GlycoLink Coupling Buffer and centrifuge. Repeat this step for a total of three washes.
8. Wash the column with 2mL of Wash Buffer and centrifuge. Repeat this step for a total of three washes.
9. Equilibrate the column for storage by adding 2mL 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 2mL of degassed buffer with 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.

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 that may impede flow into the resin bed.
- 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

A. Method

Note: Perform all centrifugations at 1000 \times g for 2 minutes using a 15mL 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 3mL of Binding/Wash Buffer and centrifuge. Repeat the equilibration once.
3. Add sample (\leq 2mL) 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). For samples > 2mL, add volumes in succession.

4. Remove top and bottom caps and centrifuge column. Without changing collection tubes, add 1mL of Binding/Wash Buffer and centrifuge again. Save the entire flow-through to evaluate binding efficiency and capacity.
5. To wash the resin, add 2mL of Binding/Wash Buffer and centrifuge. Repeat this step 2-4 times.
6. Elute the protein with 1mL of Elution Buffer collecting into a centrifuge tube containing 100µL 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 2mL of Binding/Wash Buffer to the column and centrifuge. Repeat this step once.
9. Cap the bottom and add 1mL 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 antioxidants
	Primary amines were not removed from the sample before coupling and these 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

89891	Zeba Spin Desalting Columns, 7K MWCO, 5mL
53149	UltraLink Hydrazide Resin, 10mL
88942	GlycoLink Micro Immobilization Kit
88943	GlycoLink IP Kit
88944	GlycoLink Coupling Catalyst, 100mL
20504	Sodium <i>meta</i> -Periodate, 25g
21004	IgG Elution Buffer, 1L
23260	Glycoprotein Carbohydrate Estimation Kit

General References

- O'Shannessy, D., *et al.* (1984). A novel procedure for labeling immunoglobulins by conjugation to oligosaccharide moieties. *Immunol Let* **8**:273-7.
- Domen, P., *et al.* (1990). Site directed immobilization of proteins. *J Chromatogr* **510**:293-302.
- 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.
- Dirksen, A., Dawson, PE. (2008). Rapid oxime and hydrazone ligations with aromatic aldehydes for biomolecular labeling. *Bioconjug Chem* **19** (12):2543-8.
- Byeon, J., Limpoco, F., Bailey, R. (2010). Efficient bioconjugation of protein capture agents to biosensor surfaces using aniline-catalyzed hydrazone ligation. *Langmuir* **26** (19):15430-5.

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