INSTRUCTIONS

Glycoprotein Isolation Kit, ConA

89804

1738.4

Number	Description
89804	**Glycoprotein Isolation Kit, ConA**, contains sufficient reagents for the isolation of glycoproteins with strong affinity for ConA from 10 samples of up to 640µL (1-1.5mg total protein) each

Kit Contents:

ConA Lectin Resin, 1.1mL settled resin supplied as 50% slurry

Binding/Wash Buffer, 6.5mL of a 5X stock solution

Elution Buffer, 5mL

Column Accessory Pack, 10 spin columns with bottom caps and 20 collection tubes

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

Introduction

The Thermo Scientific™ Glycoprotein Isolation Kit, ConA isolates glycoproteins from complex protein mixtures including serum and tissue and cultured cell lysates using the lectin concanavalin A (ConA) immobilized on agarose. Lectins are proteins that have a selective affinity for carbohydrate moieties. The ConA lectin preferentially recognizes α-linked mannose and, to a lesser extent, terminal glucose residues. These carbohydrates are attached to proteins through asparagine residues. Oligomannosyl saccharides are commonly present in a wide variety of serum and membrane-bound glycoproteins.

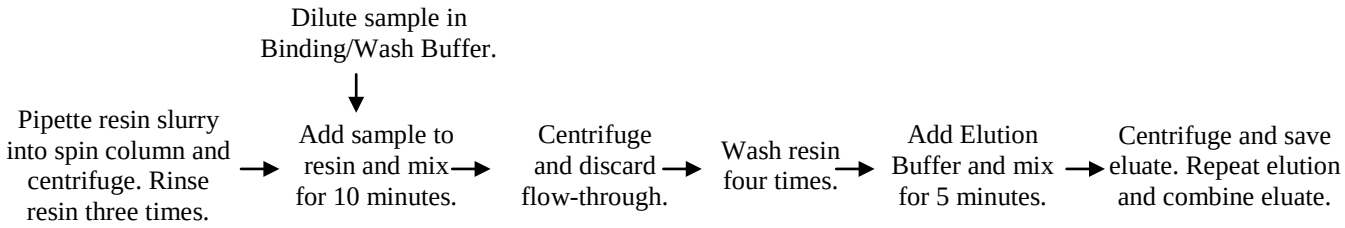
Protein glycosylation is a common post-translational modification. Asparagine (N-linked) and serine/threonine residues (O-linked) are glycosylated during passage through the endoplasmic reticulum and golgi apparatus in eukaryotic and prokaryotic (i.e., Archaea and Eubacteria) systems. Glycoconjugates are important for immune regulation, inflammation, cell-to-cell adhesion and contact inhibition, cell signaling, protection against proteolytic degradation, and other biological processes.

This kit is easy to use and contains all the necessary components for isolating most N-linked glycans. A sample containing up to 1.5mg of total protein is first diluted with the Binding/Wash Buffer and applied to the ConA resin bed. Following incubation, the resin is washed and the bound glycoproteins are eluted. Glycoproteins have been successfully isolated from serum as well as HeLa and CHO cell lysates in approximately 40 minutes.

Important Product Information

- If needed, add protease inhibitors to samples; however, avoid cocktails containing EDTA or other metal chelators.
- Samples purified with this kit are compatible with 1D gel electrophoresis and the Thermo Scientific™ Coomassie Plus™ (Bradford) Assay Kit (Product No. 23236). Many other downstream applications require sample processing to remove incompatible substances in the Elution Buffer. To quantify protein using the Thermo Scientific™ BCA Protein Assay (Product No. 23225), desalt sample using a 5mL Thermo Scientific™ Zeba™ Spin Desalting Column (Product No 89891). For 2D gel electrophoresis, remove interfering substances by precipitation or dialysis.
- For serum samples, isolation of less-abundant glycoproteins is improved by first removing the albumin and IgG. This can be accomplished with an Albumin/IgG Removal Kit (see Related Products Section).

Procedure Summary Flow Chart



Procedure for Glycoprotein Isolation using ConA

A. Preparation of sample

1. Equilibrate the Binding/Wash and Elution Buffers to room temperature.
2. Dilute sample containing 1 to 1.5mg of total protein 4:1 with 5X Binding/Wash Buffer stock solution (e.g., mix 400µL sample with 100µL 5X Binding/Wash Buffer). The total volume, including dilution, must not exceed 800µL.

B. Isolation of glycoproteins

1. To prepare the 1X Binding/Wash Buffer dilute 460µL 5X Binding/Wash Buffer with 1840µL of ultrapure water, which is sufficient volume to process one sample.
2. Insert a column into a collection tube.
3. Gently swirl the bottle of ConA Lectin Resin to obtain a homogeneous suspension. Use a wide-bore or cut pipette tip to transfer 200µL of 50% resin slurry to the column.
4. Centrifuge 1 minute at 1000 × *g* and discard the storage buffer. Reuse the collection tube through Step B12.
5. Place column in collection tube and add 200µL 1X Binding/Wash Buffer to the resin. Close the top cap and centrifuge column for 1 minute at 1000 × *g* and discard rinse. Repeat this step two times.
6. Place bottom cap on column and add sample, from Step A1, to the resin. Close the top cap.
7. Incubate column for 10 minutes at room temperature with end-over-end mixing using a rotator (e.g., Labquake™ Shaker by Thermolyne). Alternatively, rock back and forth on a rocking platform.
8. Remove top cap and then bottom cap from column. Place column in the collection tube, and replace top.

   Note: Remove top cap before bottom cap to prevent sample from leaking from the bottom of the column.
9. Centrifuge column for 1 minute at 1000 × *g* and discard flow-through.

   Note: If desired, save flow-through for SDS-PAGE or protein assay analysis.
10. Reinsert column and add 400µL 1X Binding/Wash Buffer to the resin. Cap column and centrifuge for 1 minute at 1000 × *g* and discard flow-through. Repeat this step.
11. Place bottom cap on column and add 400µL 1X Binding/Wash Buffer to the resin. Cap column and incubate for 5 minutes at room temperature with end-over-end mixing using a rotator.
12. Remove top cap and then bottom cap from column. Place column in the collection tube, and replace top cap. Centrifuge column for 1 minute at 1000 × *g* and discard rinse.
13. Repeat Steps B11-B12.
14. Replace bottom cap on column. Add 200µL Elution Buffer to resin and cap column. Incubate column for 5 minutes at room temperature with end-over-end mixing using a rotator.
15. Remove top cap and then bottom cap from column. Place column in a new collection tube. Replace top cap and centrifuge column for 1 minute at 1000 × *g*.
16. Carefully set aside the collection tube and remove top cap.
17. Repeat Steps B14-B16. Collect eluate in the same collection tube containing eluate from the first elution. Store eluted glycoproteins on ice for immediate use or freeze for later analysis.

Troubleshooting

Problem	Possible Cause	Solution
Low glycoprotein recovery in elution fraction	Some glycoproteins have high affinity for the immobilized ConA and will not elute with Elution Buffer	Increase incubation with elution buffer to 10 minutes or boil resin in 200µL of SDS-PAGE sample buffer for 5 minutes and then centrifuge column in a 2mL tube for 1 minute at 1000 × *g* to collect eluate **Note:** Boiling the resin results in detachment of some lectin and also may release nonspecifically bound proteins.
Glycoprotein is not binding to the resin	Sample contains metal chelator(s)	Confirm EDTA or other metal chelators are not present in the sample

Related Thermo Scientific Products

89805 **Glycoprotein Isolation Kit, WGA**

89875 **Pierce Albumin/IgG Removal Kit**

89161 **Top 2 Abundant Protein Depletion Columns (Human Albumin and IgG)**

89891 **Zeba Spin Desalting Columns**

23225 **BCA Protein Assay Kit**

23236 **Coomassie Plus (Bradford) Assay Kit**

General References

Cooper, C.A., *et al.* (2001) GlycoSuiteDB: A new curated relational database of glycoprotein glycan structures and their biological sources. *Nucl Acid Res* **20(1):**332-5.

Cummings, R.D. (1997). Affinity chromatography of oligosaccharides and glycopeptides. Affinity Separations: A Practical Approach (Matejschuk, P., Ed.), Oxford Univ. Press, London, pp. 123-39.

Ghosh, D., *et al*. (2004). Lectin affinity as an approach to the proteomic analysis of membrane glycoproteins. *J Proteome Res* **3:**841-50.

Young, N.M., *et al.* (2002). Structure of the N-linked glycan present on multiple glycoproteins in the gram-negative bacterium, *Campylobacter jejuni*. *J Biol Chem* **277:**42530-9.