INSTRUCTIONS

Glycoprotein Isolation Kit, WGA

PIERCE
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89805

1755.0

Number	Description
89805	**Glycoprotein Isolation Kit, WGA**, contains sufficient reagents for the isolation of glycoproteins with strong affinity for WGA from 10 samples of up to 640 µl (1-1.5 mg total protein) each
	Kit Contents:
	WGA Lectin Resin, 1.1 ml settled resin supplied as a 50% slurry containing a hapten buffer
	Binding/Wash Buffer, 6.5 ml of a 5X stock solution
	Elution Buffer, 5 ml
	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 Glycoprotein Isolation Kit, WGA isolates glycoproteins from complex protein mixtures including serum and tissue and cultured cell lysates using the lectin wheat germ agglutinin (WGA) immobilized on agarose. Lectins are proteins that have a selective affinity for carbohydrate moieties. The WGA lectin preferentially binds N-acetyl glucosamine (GlcNAC) and terminal GlcNAC structures that are commonly present in many serum and membrane glycoproteins. WGA also has affinity for sialic acid.

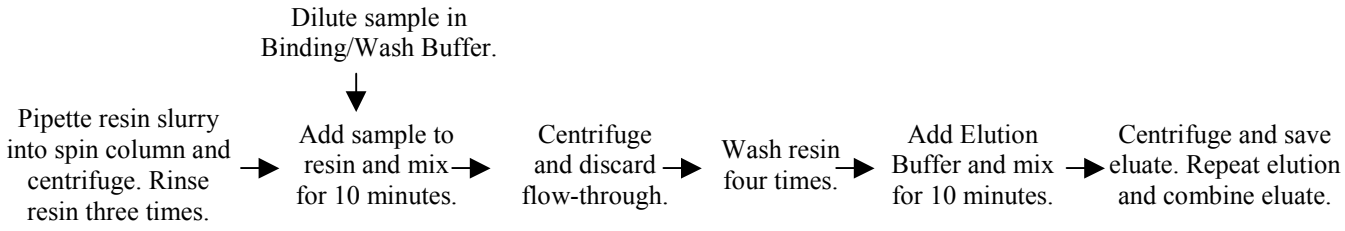
Protein glycosylation is a common post-translational modification. Asparagine (N-linked) and serine/threonine residues (O-linked) are glycosylated during passage through the endoplasmic reticulum and golgi apparatus in eukaryotic and prokaryotic (i.e., Archaea and Eubacteria) systems. Glycoconjugates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

This kit is easy to use and contains all the necessary components for isolating sialic acid/GlcNAC-containing glycans. A sample containing up to 1.5 mg of total protein is first diluted with the Binding/Wash Buffer and applied to the WGA 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 50 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 1-D gel electrophoresis and the Coomassie Plus – The Better 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 BCA™ Protein Assay (Product No. 23225), desalt sample using a 5 ml Zeba™ Desalt Column (Product No 89891). For 2-D gel electrophoresis, clean up sample using the 2-D Sample Prep for Soluble (Product No. 89865) or Insoluble Proteins (Product No. 89866) kit. Alternatively, remove interfering substances by precipitation or dialysis.
- Removing albumin and IgG from serum samples improves isolation of less-abundant glycoproteins. If desired, use the ProteoSeek™ Albumin/IgG Removal Kit (Product No. 89875) before glycoprotein isolation to deplete these abundant proteins from serum samples.

Procedure Summary Flow Chart



Procedure for Glycoprotein Isolation using WGA

A. Preparation of sample

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

B. Isolation of glycoproteins

1. To prepare the 1X Binding/Wash Buffer, dilute 460 µl 5X Binding/Wash Buffer with 1,740 µl 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 WGA Lectin Resin to obtain a homogeneous suspension. Use a wide-bore or cut pipette tip to transfer 200 µl of 50% resin slurry to the column.
4. Centrifuge 1 minute at 1,000 × *g* and discard the storage buffer. Reuse the collection tube through Step B12.
5. Place column in collection tube and add 200 µl 1X Binding/Wash Buffer to the resin. Close the top cap and centrifuge column for 1 minute at 1,000 × *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 1,000 × *g* and discard flow-through.

   Note: If desired, save the flow-through for SDS-PAGE or protein assay analysis.
10. Reinsert column and add 400 µl 1X Binding/Wash Buffer to the resin. Cap column and centrifuge for 1 minute at 1,000 × *g* and discard flow-through. Repeat this step.
11. Place bottom cap on column and add 400 µl 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 1,000 × *g* and discard flow-through.
13. Repeat Steps B11-B12.
14. Replace bottom cap on column. Add 200 µl Elution Buffer to resin and cap column. Incubate column for 10 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 1,000 × *g*.
16. Carefully set aside the collection tube and remove top cap.
17. Repeat Steps B14-B16. Collect eluate in the same collection tube that contains 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 WGA and will not elute with Elution Buffer	Increase incubation with elution buffer to 15 minutes, or boil resin in 200 µl of SDS-PAGE sample buffer for 5 minutes and then centrifuge column in a 2 ml tube for 1 minute at 1,000 × *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 Pierce Products

89804 **Glycoprotein Isolation Kit, ConA**

78415 **Halt™ Protease Inhibitor Cocktail Kit, EDTA-Free**

89891 **Zeba™ Desalt Spin Columns,** 5 ml

89865 **2-D Sample Prep for Soluble Proteins**

89866 **2-D Sample Prep for Insoluble Proteins**

89875 **ProteoSeek™ Albumin/IgG Removal Kit**

66335 **Slide-A-Lyzer® Dialysis Cassette Kit, 3.5 MWCO, 0.1-0.5 ml**

23225 **BCA™ Protein Assay Kit**

23236 **Coomassie Plus – The Better 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. 123-139.

Ghosh, D., *et al.* (2004). Lectin affinity as an approach to the proteomic analysis of membrane glycoproteins. *J. Proteome Res.* **3:**841-850.

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.

Current versions of all product instructions are available at *www.piercenet.com.* For a faxed copy call 800-874-3723 or contact your local distributor.