Glycoprotein Enrichment Resin User Manual

Cat. No. 635647
PT4050-1 (PR912675)
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I. Introduction

Glycoprotein Enrichment Resin (Cat. No. 635647) is a phenylboronic acid-based resin which provides quick, efficient, and specific enrichment of glycoproteins from complex samples such as human serum, using either simple gravity flow columns or medium pressure methods such as FPLC (*Clontechniques,* July 2008). The resin consists of an *m*-aminophenylboronic acid ligand (Brena *et al.*, 1992) coupled to agarose beads. The ligand binds to *cis*-diol groups on sugar residues such as mannose, galactose, or glucose that are present within the saccharide moiety of glycoprotein molecules, forming a reversible five-member ring complex (Figure 1). This complex can be dissociated by lowering the pH, or by using an elution buffer containing either Tris or sorbitol.

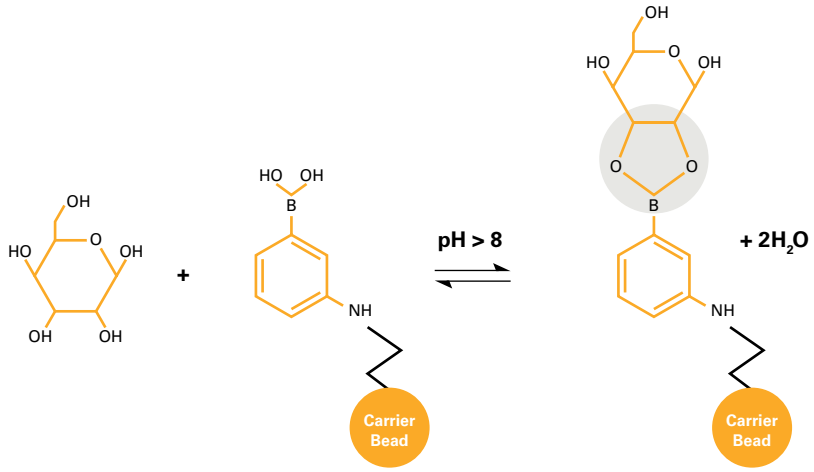

Figure 1. Molecular mechanism of saccharide binding to Glycoprotein Enrichment Resin.

Specific Enrichment of Glycoproteins

Glycoprotein Enrichment Resin facilitates the identification of specific glycoproteins using downstream applications such as mass spectroscopy—by enriching these proteins from complex mixtures containing both nonglycosylated and glycosylated proteins. The resin removes more abundant nonglycosylated proteins such as serum albumin that can mask the presence of glycoproteins, as well as saline contaminants that interfere with ionization.

II. List of Components

Store all components at 4°C.

Glycoprotein Enrichment Resin (Cat. No. 635647)

- Glycoprotein Enrichment Resin

Other

- User Manual (PT4050-1)

Visit our Protein Expression and Purification product pages at **www.clontech.com** for a current list of products available for protein research.

III. Additional Materials Required

A. Buffers

These buffers have been optimized for enrichment of glycoproteins from human serum using Glycoprotein Enrichment Resin.

- **Binding/Wash Buffer**
  (50 mM HEPES, 0.5 M NaCl, pH 8.0–8.5)

NOTE: If required, add 20–50 mM Mg^{2+} to this buffer, depending on the protein. Please note that although adding Mg^{2+} may increase the binding capacity of the resin, it can also increase nonspecific binding. Thus, the Mg^{2+} concentration may need to be optimiized for specific applications.

- **Elution Buffer**
  (100 mM sorbitol, 50 mM HEPES, 0.5 M NaCl, pH 8.0–8.5) or
  (100 mM Tris, 50 mM HEPES, 0.5 M NaCl, pH 8.0–8.5)

B. Columns

- Either gravity-flow or FPLC columns may be used.
- TALON 2-ml Disposable Gravity Columns (Cat. No. 635606) are recommended.

IV. Glycoprotein Enrichment

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING
This procedure has been optimized for glycoprotein enrichment from human serum using (A) gravity-flow columns or (B) batch/gravity flow column purification.

Protocol
90 min

A. PROTOCOL: Glycoprotein Enrichment Using a Gravity-Flow Column

1. Place the empty column on a stand in an upright position.
2. Load and run 1 ml of deionized water through the column to wet the filter.
3. Ensure that the column is plugged with a stopper.
4. Thoroughly resuspend Glycoprotein Enrichment Resin and transfer 2 ml of suspension (1 ml of resin) to the column. Allow resin to settle.
5. Remove the stopper and allow the storage buffer to flow through the column.
6. Wash the column with 5 bed-volumes of water.
7. Equilibrate the column by washing with ≥10 column-volumes of Binding/Wash Buffer. Check the pH of the effluent to make certain it is in the range of 8.0–8.5.

NOTE: In order to achieve binding of certain glycosylated proteins, it may be necessary to add 20–50 mM Mg^{2+}. to the buffer.

8. Dilute 50–100 µl of serum sample to a total volume of 2.5 ml with Binding/Wash Buffer.
9. Put the bottom stopper on the column and add the diluted serum sample. (Retain 50–100 µl of diluted sample if required for analysis.)
10. Put the top cap on and mix for 20 min on a shaker at room temperature.
11. Return the column to a stand in a upright position.
12. Collect the flowthrough.
13. Wash the column 4X with 5 ml of Binding/Wash Buffer.
14. Elute the protein with five separate 1 ml aliquots of Elution Buffer.
15. Determine the concentration of each of the five eluted fractions using a Bradford assay, or by measuring the absorbance at 280 nm.

NOTES:

- Use the Bradford assay for determining protein concentration, since the presence of sorbitol or Tris in the elution buffer will interfere with the BCA protein assay.
- The second & third fractions should contain the maximum amount of glycosylated protein.

IV. Glycoprotein Enrichment continued

Protocol
90 min

B. PROTOCOL: Glycoprotein Enrichment Using Batch/Gravity-Flow Column Purification

A hybrid batch/gravity flow column procedure can be used for purification. In this hybrid procedure, the binding and initial washing steps are performed in a batch format to save time, eliminate extraneous debris, and avoid column clogging. After the initial washes, the resin is transferred to a column for additional washing and protein elution.

1. Thoroughly resuspend the Glycoprotein Enrichment Resin.
2. Immediately transfer the required amount of resin suspension to a sterile tube that will accomodate 10–20X the resin bed volume.
3. Centrifuge at 700 x g for 2 min to pellet the resin.
4. Remove and discard the supernatant.
5. Add 10 bed-volumes of Binding/Wash Buffer and mix briefly to pre-equilibrate the resin.

NOTE: In order to achieve binding of certain glycosylated proteins, it may be necessary to add 20–50 mM Mg^{2+}. to the buffer.

6. Recentrifuge at 700 x g for 2 min to pellet the resin. Discard the supernatant.
7. Repeat Step 6.
8. Add the serum sample diluted with Binding/Wash Buffer (pH 8.0–8.5) to the resin.
9. Gently agitate at room temperature for 20 min on a shaker to allow the glycosylated proteins to bind to the resin.
10. Centrifuge at 700 x g for 5 min.
11. Carefully remove as much supernatant as possible without disturbing the resin.
12. Wash the resin by adding 10–20 bed-volumes of Binding/Wash Buffer. Gently agitate the suspension at room temperature for 5 min to promote thorough washing.
13. Centrifuge at 700 x g for 5 min.
14. Remove and discard the supernatant.
15. Repeat steps 12–14.
16. Add one bed-volume of Binding/Wash Buffer to the resin and resuspend by vortexing.
17. Transfer the resin to a 2 ml gravity-flow column with an end-cap in place, and allow the resin to settle out of suspension.
18. Remove the end-cap, and allow the buffer to drain out until it reaches the top of the resin bed, making sure no air bubbles are trapped in the resin bed.
19. Wash column once with 5 bed-volumes of Binding/Wash Buffer.
20. Elute the glycosylated proteins by adding 5 bed-volumes of Elution Buffer to the column. Collect the eluates in 1 ml fractions.

NOTE: Under most conditions, the majority of the glycosylated proteins will be eluted in the first three bed-volumes.

21. Use a Bradford protein assay or UV absorbance at 280 nm to determine the protein concentration of the fractions.

V. Troubleshooting Guide

Table I. Troubleshooting Guide for Glycoprotein Enrichment Resin

Description of Problem	Explanation	Solution
The column becomes clogged.	Debris from the sample has clogged the column.	1. Use the Batch/Gravity Flow Protocol (Section IV.B). 2. Dilute the sample with Binding/Wash Buffer and filter using a syringe filter before loading on the column.
Glycosylated proteins do not bind to the column at all.	1. The sample pH does not fall within the desired range of 8.0–8.5.	1. Check the pH of the sample and the Binding/Wash Buffer. If the sample pH is not within the desired range and the buffer pH is correct, then dilute the sample with the buffer.
	2. Sugars present in the sample compete for binding.	2. Remove low molecular weight sugars by desalting on a PD-10 column (GE Healthcare).
Certain glycosylated proteins bind poorly to the column.	Additional Mg^{2+} is needed to optimize binding.	Add 20–50 mM Mg^{2+} to the Binding/Wash Buffer.

VI. References

Brena, B. M. *et al.* (1992) *J. Chromatogr.* **604**(1):109–115.

Glycoprotein Enrichment & Detection (July 2008) *Clontechniques* **XXIII**(3):6–8.

Appendix A: Sample Results and Analysis

Glycoprotein Enrichment Resin can be used to enrich even low-abundance serum glycoproteins, such as alpha-1 antitrypsin and alpha-1-acid glycoprotein (Figure 2), as confirmed by Western blot analysis using antibodies specific for each of these proteins.

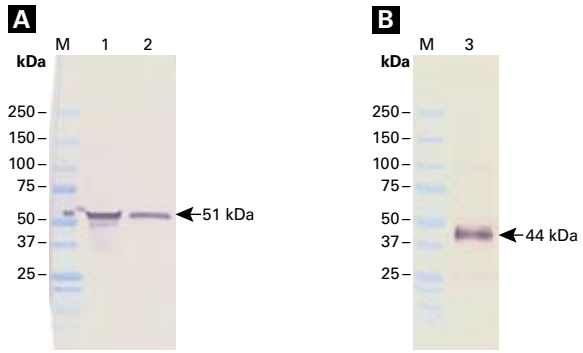

Figure 2. Glycoprotein Enrichment Resin enriches specific serum glycoproteins. Enriched fractions from the column containing Clontech's Glycoprotein Enrichment Resin were analyzed by Western blotting with antibodies for two specific serum glycoproteins, alpha-1 acid glycoprotein (**Panel A**) and alpha-1 antitrypsin (**Panel B**). Lane 1: human serum. Lane 2: eluted fraction. Lane 3: eluted fraction. As seen in Lane 2 (**Panel A)** and Lane 3 (**Panel B**), both alpha-1 acid glycoprotein (51 kDa) and alpha-1 antitrypsin (44 kDa) bound to the column, though some loss was observed in the flowthrough (not shown).