Glycoprotein Enrichment and Detection

Quick, easy phenylboronic acid-based resin enrichment & Western detection of glycoproteins

- **Efficient, specific enrichment of glycoproteins**
- **Use with gravity flow or FPLC columns—for better results in downstream applications**
- **Rapid, selective detection of glycoproteins on Western blots**

Clontech's new **Glycoprotein Enrichment Resin** specifically enriches glycoproteins from complex samples such as human serum, using either simple gravity flow columns or medium pressure chromatography such as FPLC—with higher specificity and capacity than a competitor's resin. Enriched glycoproteins transferred to membranes for Western blotting are selectively detected using our new **Glycoprotein Western Detection Kit**. It provides highly sensitive and rapid detection—yielding results within one hour, in less time and fewer steps than a competing kit.

Simple, Phenylboronic Acid-Based Enrichment

Glycosylated proteins, which comprise 50–60% of proteins in the human body, represent the majority of cell surface markers and secreted proteins. In order to identify specific glycoproteins using downstream applications such as mass spectroscopy, the proteins must be separated from complex mixtures that contain both nonglycosylated and glycosylated proteins.

Reduction of sample complexity can be achieved using Glycoprotein Enrichment Resin, which consists of an *m*-aminophenylboronic acid ligand (1) 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.

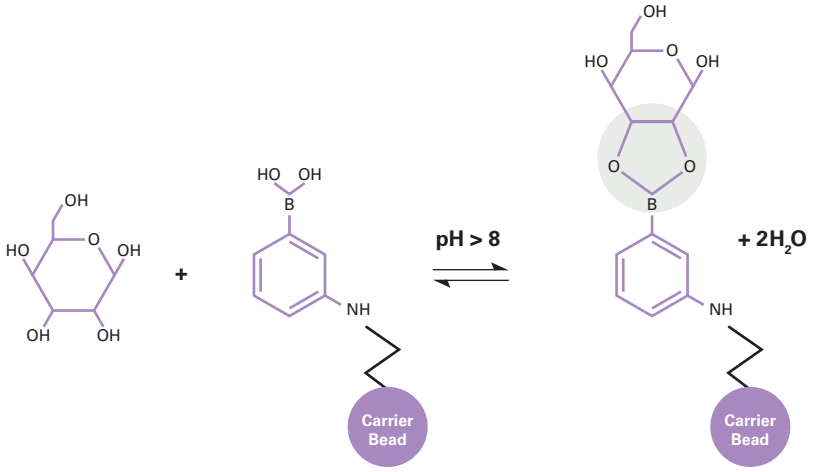

Figure 1. Molecular mechanism of saccharide binding to Clontech's Glycoprotein Enrichment Resin. An *m*-aminophenylboronic acid ligand coupled to agarose beads binds to *cis*-diol groups on sugar residues such as mannose, galactose, or glucose that are present within the saccharide moiety of glycoprotein molecules, forming a reversible five-member ring complex. This complex can be dissociated by lowering the pH or by using an elution buffer containing either Tris or sorbitol.

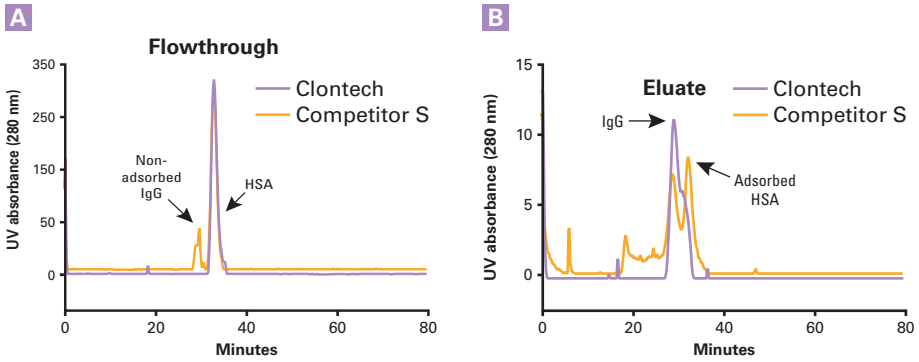

Figure 2. Clontech's Glycoprotein Enrichment Resin binds serum glycoproteins more specifically than a competitor's resin. A gravity flow column was packed with 1 ml of Glycoprotein Enrichment Resin, and washed with water. After equilibration with Loading Buffer (50 mM HEPES, 0.5 M NaCl, pH 8.5), 50 µl of human serum diluted to 2.5 ml was incubated with the resin in the column for 20 min, and the column was washed with Loading Buffer. Bound proteins were eluted with 100 mM Tris in Loading Buffer. A second column packed with Competitor S resin was used to enrich another 50 µl of human serum using the buffers and conditions specified in the accompanying protocol. All fractions were analyzed by FPLC on a gel filtration column (Superdex® 200HR 10/30). **Panel A** compares the flowthrough fractions from the column containing Clontech's resin with those from the Competitor S column, while **Panel B** compares the eluted glycoproteins. The flowthrough fraction from Clontech's resin displayed only nonglycosylated HSA (human serum albumin), while the enriched fraction contained mainly IgG (glycoprotein). In contrast, the flowthrough fractions obtained from the Competitor S resin exhibited a loss of IgG, while the eluate displayed adsorption of HSA in addition to IgG, indicating nonspecific binding of nonglycosylated HSA.

Glycoprotein Enrichment and Detection...continued

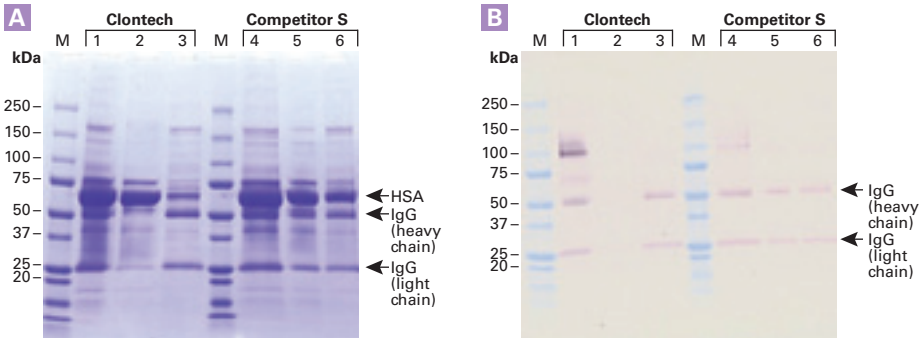

Figure 3. SDS-PAGE and Western blot analysis of Clontech's Glycoprotein Enrichment Resin column fractions demonstrate more effective glycoprotein enrichment than a competitor's resin. Panel A. Fractions from the gravity flow enrichment procedures described in Figure 2 were analyzed by SDS-PAGE. As seen in the Coomassie-stained gel, the Competitor S Resin showed binding of HSA (human serum albumin) to the column (Lane 6) and leakage of IgG in the flowthrough fraction (Lane 5). **Panel B.** Fractions from the column were also analyzed by Western blotting with an antibody to human IgG. The Clontech resin retained glycosylated IgG (Lane 3), with no visible loss of IgG in the flowthrough fraction (Lane 2). In contrast, the Competitor S Resin exhibited a loss of IgG in the flowthrough fraction (Lane 5). Lane M: molecular weight marker. Lanes 1, 4: human serum. Lanes 2, 5: flowthrough. Lanes 3, 6: eluate (glycoproteins).

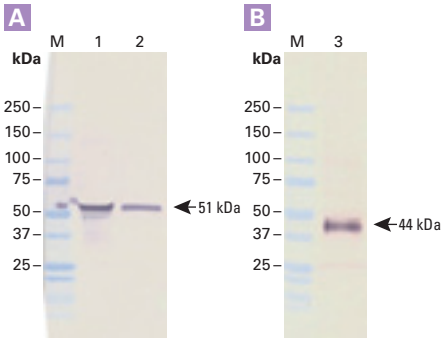

Figure 4. Glycoprotein Enrichment Resin enriches specific serum glycoproteins. Enriched fractions from the column containing Clontech's Glycoprotein Enrichment Resin were analyzed by Western blotting with antibodies for two specific serum glycoproteins, alpha-1 acid glycoprotein (**Panel A**) and alpha-1 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).

Phenylboronic acid-based resins also facilitate the enrichment of proteins containing the more heterogeneous O-linked oligosaccharides, as well as those subject to nonenzymatic glycosylation (i.e., glycation). Thus, this resin may be used to enrich a broader range of glycoproteins than affinity resins, which use lectins or antibodies that are specific for certain glycan modifications. Glycoprotein Enrichment Resin is ideal for reducing sample complexity in order to study a variety of different serum proteins, since it increases the overall sensitivity of downstream applications to glycoproteins.

More Specific than the Competition

Glycoprotein Enrichment Resin was used to enrich glycosylated proteins from human serum. A column containing the resin retained IgG, the most abundant glycosylated protein, but exhibited minimal nonspecific binding of human serum albumin, the most abundant nonglycosylated serum protein. Our resin was compared side by side with a boronic acid affinity resin from Competitor S for enrichment of glycoproteins from human serum. As shown in Figure 2, Panel B, enriched sample from Competitor S contained a higher concentration of nonglycosylated human serum albumin (HSA) than IgG (glycoprotein), while Clontech's resin enriched mainly IgG. Moreover, the flowthrough fraction from the Competitor S resin contained IgG that was not retained by this resin (Figure 2, Panel A). These results were confirmed by SDS-PAGE (Figure 3, Panel A) and Western blot analysis (Figure 3, Panel B).

Effectively Enriches Low-Abundance Glycoproteins

Glycoprotein Enrichment Resin provides a simple and effective means for enriching low-abundance glycoproteins for further study. The specific binding of two low-abundance serum glycoproteins, alpha-1 antitrypsin and alpha-1-acid glycoprotein, to Glycoprotein Enrichment Resin, was confirmed by Western blot analysis (Figure 4) using antibodies specific for each of these proteins.

Glycoproteins are important in disease diagnosis, as well as proteomics. However, they can be masked by the presence of more abundant nonglycosylated proteins such as serum albumin, making them difficult to detect and analyze using downstream applications such as mass spectroscopy. Moreover, direct detection via mass spectroscopy from samples such as blood serum, urine, and spinal fluid can be inhibited by saline contaminants which interfere with ionization. Clontech's Glycoprotein Enrichment Resin provides a powerful tool for specific enrichment of glycoproteins and removal of saline contaminants from complex samples in order to facilitate downstream analysis.

Glycoprotein Enrichment and Detection...continued

Rapid, Sensitive Western Detection of Glycoproteins

The Glycoprotein Western Detection Kit, an accessory product to our Glycoprotein Enrichment Resin, is designed for selective staining of glycoproteins on Western blots. The kit uses a modification of the periodic acid-Schiff method (2) to stain glycoprotein carbohydrate moieties, yielding colored magenta bands. The periodic acid-Schiff reagent, which stains vicinal diol groups found mainly on peripheral sugars and sialic acids, is commonly used as a general glycoprotein stain (3).

Product	Size	Cat. No.
Glycoprotein Enrichment Resin	10 ml	635647
Glycoprotein Western Detection Kit	20 rxns	635648
TALON 2-ml Disposable Gravity Columns	50 columns	635606

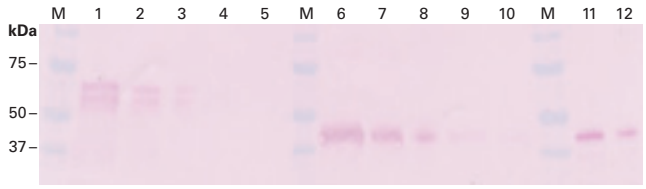

Figure 5. Highly sensitive detection of purified glycoproteins with Clontech's Glycoprotein Western Detection Kit. Varying amounts of three different purified glycoproteins [fetuin (Lanes 1–5), alpha-1 acid glycoprotein (AGP; Lanes 6–10), and horseradish peroxidase (HRP; Lanes 11 & 12)] were electrophoresed on a 4–15% SDS-PAGE gel, transferred to a nitrocellulose membrane, and detected using the Glycoprotein Western Detection Kit. Lane M: molecular weight marker. Lane 1: 1 µg fetuin. Lane 2: 500 ng fetuin. Lane 3: 200 ng fetuin. Lane 4: 100 ng fetuin. Lane 5: 50 ng fetuin. Lane 6: 1 µg AGP. Lane 7: 500 ng AGP. Lane 8: 200 ng AGP. Lane 9: 100 ng AGP. Lane 10: 50 ng AGP. Lane 11: 350 ng HRP. Lane 12: 140 ng HRP.

Figure 6. Selective glycoprotein detection using Clontech's Glycoprotein Western Detection Kit. Varying amounts of purified horseradish peroxidase (HRP), a known glycoprotein (Lanes 1–4), and soybean trypsin inhibitor (SBTI), a nonglycosylated protein (Lanes 5–8), were electrophoresed on a 4–15% SDS-PAGE gel and transferred to a nitrocellulose membrane. Lane M: molecular weight marker. Lane 1: 700 ng HRP. Lane 2: 350 ng HRP. Lane 3: 140 ng HRP. Lane 4: 70 ng HRP. Lane 5: 700 ng SBTI. Lane 6: 350 ng SBTI. Lane 7: 140 ng SBTI. Lane 8: 70 ng SBTI. Glycoprotein bands were specifically detected in Lanes 1–4, but not in Lanes 5–8.

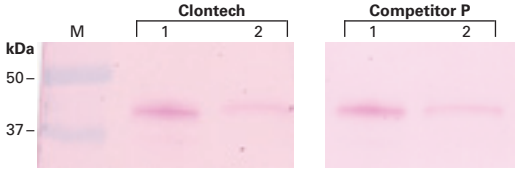

Figure 7. Comparison of detection sensitivities between Clontech's Glycoprotein Western Detection Kit and a competing kit. Varying amounts of purified horseradish peroxidase (HRP) were electrophoresed on a 4–15% SDS-PAGE gel and transferred to a nitrocellulose membrane. Glycoprotein bands were detected using both the Glycoprotein Western Detection Kit and a kit from Competitor P, according to their respective user manuals. Lane M: molecular weight marker. Lane 1: 350 ng HRP. Lane 2: 140 ng HRP. Clontech's Glycoprotein Western Detection Kit delivers equivalent sensitivity in fewer steps.

Related Products

- Phosphoprotein Enrichment Kit (Cat. No. 635624)
- TALON® PMAC Magnetic Phospho Enrichment Kit (Cat. No. 635641)
- Thiophilic-Superflow™ Resin (Cat. Nos. 635616 & 635617)
- Mag-Trypsin (Cat. No. 635646)
- TALON® Metal Affinity Resin (Cat. Nos. 635501, 635502, 635503 & 635504)

Our kit allows highly sensitive (Figure 5) and selective (Figure 6) detection of glycoproteins that have previously been transferred to Western membranes—in about 1 hour. It provides faster results in fewer steps—and is as sensitive as other kits currently on the market (Figure 7).

Clontech's Glycoprotein Enrichment Resin and Glycoprotein Western Detection Kit offer simple, efficient, and highly specific glycoprotein analysis tools to meet your research needs.

References

1. Brena, B. M. *et al.* (1992) *J. Chromatogr.* **604**(1):109–115.
2. Zacharius, R. M. *et al.* (1969) *Anal. Biochem.* **30**(1):148–52.
3. Thornton, D. J. *et al.* (1994) *Methods Mol. Biol.* **32**:119–28.