BIOTECH SUPPORT GROUP

# NuGel™ Glycoprotein Enrichment PBA Kit

*With Phenyl Boronic Acid NuGel™ Matrix*

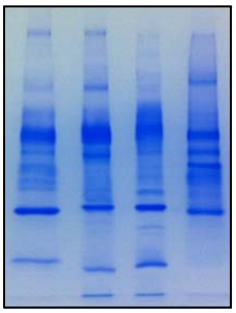
- Cis-diol specific, enriches heterogeneous sets of glycoproteins
- Unmasks glycoproteins from high abundance proteins, most notably albumin
- Disposable, no column regeneration or cross-contamination
- Efficient new surface technology ideal for proteomic applications
- Enriches glycoproteins from blood, serum, plasma, tissue or cell culture media.
- Removes > 90% of serum albumin (the non-glycosylated fraction)
- Sorbitol elution at pH 7.5 – 8.5

Silica has been an industry standard as an advantageous matrix suitable for high performance liquid chromatography. With NuGel™, non-specific sites have been virtually eliminated making it an ideal support for affinity purification. Through a proprietary polymer coating, silica is crosslinked forming a reactive Poly-Epoxy functionality stable across a wide pH range (pH 2 to 10). From this foundational chemistry, all of the NuGel™ affinity products are derived.

The Phenyl Boronic Acid (PBA) ligand is immobilized through the NuGel™ poly-Epoxy linkage with attachment through the amino group. While various lectins bind to specific saccharide residues, the PBA ligand binds to the 1,2-cis-diol groups of biomolecules and enriches for heterogeneous sets of glycoproteins containing both N-linked and O-linked oligosaccharides. An easy and fast spin-filter format makes glycoprotein enrichment simple starting from 50µl serum, or 1-2 mg total protein.

| SAMPLE | % Glycoprotein Eluted with Sorbitol |
|---|---|
| Mouse Plasma | 33 |
| Rat Serum | 44 |
| Sheep Serum | 18 |
| Bovine Serum | 40 |
| Bovine Brain Homogenate | 9 |

SDS-PAGE, 4-15% Tris-HCl



A B C D

Different heterogeneous sets of glycoproteins are observed from 4 different mammalian

**Gel Key**:

**A**: Mouse Plasma Eluate

**B**: Sheep Serum Eluate

**C**: Bovine Serum Eluate

**D**: Rat Serum Eluate

| Product | Size | Total samples processed | Item No. | Price |
|---|---|---|---|---|
| **NuGel™ Glycoprotein Enrichment PBA Kit** | 10 Preps | 10X 50 µl of Serum or Plasma (1-2mg total protein each prep) | NGPBA-10 | $305 |
| **NuGel™ Glycoprotein Enrichment PBA Kit** | 50 Preps | 50X 50 µl of Serum or Plasma (1-2mg total protein each prep) | NGPBA-50 | $1150 |
| **Note: Please contact sales@biotechsupportgroup.com for prices in bulk amount.** | | | | |

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| Items | 10 Prep | 50 Prep | Reagent |
|---|---|---|---|
| NuGel™ Glycoprotein Enrichment PBA Matrix, NGPBA | 0.5 gram | 2.5 grams | Supplied |
| Binding Buffer NGPBA-BB, *PH 8.5* | 5 ml | 25 ml | Supplied |
| Wash Buffer NGPBA-WB, *PH 8.5* | 15 ml | 75 ml | Supplied |
| Elution Buffer NGPBA-EB, *PH 8.5* | 5 ml | 25 ml | Supplied |
| SpinX Centrifuge tube filters | 10 | 50 | Supplied |

## PROTOCOL – Based on processing 50 µl Serum or 1-2 mg total protein

1. Weigh out 50 mg of **NuGel™ Glycoprotein Enrichment PBA matrix, NGPBA** into the supplied spin-filter. Tap to ensure reagent goes down to the filter.

2. Add 250 µl of **Binding Buffer NGPBA-BB** to the spin-filter. Vortex for 5 minutes and centrifuge for 3 minutes at 10,000 rpm. Discard the filtrate (Flow-Through).

3. Condition the sample by adding 200 µl of **Binding Buffer NGPBA-BB** to 50 µl of serum (or up to 200 µl of clarified tissue lysates). Vortex for 10 minutes and then centrifuge for 3 minutes at 10,000 rpm and discard the filtrate (Flow-Through).

4. Add 350 µl of **Wash Buffer NGPBA-WB**. Vortex for 5 minutes then centrifuge for 2 minutes at 10,000 rpm. Repeat this step 2 times. **The bead is now enriched with glycoproteins. For on-bead digestion for LC-MS work see on-bead digestion protocol, otherwise proceed to the next step.**

5. Add 300 µl of **Elution Buffer NGPBA-EB**. Vortex for 10 minutes and centrifuge for 3 minutes at 10,000 rpm. The Eluate contains the glycoprotein fraction. The eluate is ready for further functional or LC-MS studies.

Note:

- The protocol can be scaled up or down proportionally to adjust for different serum volumes. The surface amount can be adjusted to accommodate more or less albumin removal.
- We have 0.45µ SpinX centrifuge tube filters. If required can be ordered separately.

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## Suggested On-Bead Digestion Protocol

- After the final wash steps from step 7, add 100 µls of 5 mM DTT solution to the beads for complete immersion, mix and incubate at 60°C for ½ hour.
- After cooling, add 100 µls of 25 mM iodoacetamide to the DTT/bead suspension, mix and incubate in the dark for 1 hour.
- Centrifuge at 5000xg (medium setting, not max) for 3 mins, and discard filtrate. Transfer the filter slurry of beads, DTT and iodoacetamide to a clean Eppendorf tube.
- On-bead digestion is done by adding 100 µls of a 0.025 ug/uL solution of MS-grade. Trypsin to the beads. Digest overnight at 37°C.
- Centrifuge at 5000xg (medium setting, not max) for 3 mins, and retain peptide filtrate.
- To further extract remaining peptides, add 100 µls of 10% solution of formic acid to the beads.
- Incubate for 15 minutes at 37°C, centrifuge at 5000xg (medium setting, not max) for 3 mins, and add this volume to the first volume.
- Reduce to a final volume of 100 µls using a SpeedVac and store at -80 °C until LC-MS/MS.

## RELATED PRODUCTS:

- AlbuVoid™ - Albumin Depletion and Low Abundance Protein Enrichment Kit from Serum or Plasma
- Cleanascite™ Lipid Removal Reagent and Clarification
- HemogloBind™ Hemoglobin Depletion From Hemolyzed Serum/Plasma
- Corning® Spin-X Filter

## CONTACT US

We welcome your questions and comments regarding our products.


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