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NuGel™ Glycoprotein Enrichment PBA Kit

With Phenyl Boronic Acid NuGel™ Beads

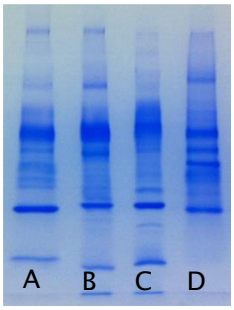
- Cis-diol specific, enriches heterogeneous sets of glycoproteins
- Unmasks glycoproteins from high abundance proteins, most notably albumin
- Disposable, no column regeneration or cross-contamination
- Efficient new surface technology ideal for proteomic applications
- Enriches glycoproteins from blood, 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



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

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

Items	10 Prep	50 Prep	Reagent
NuGel™ Glycoprotein Enrichment PBA Beads, 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
Spin-Filter/tube assemblies	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 beads, 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, see on-bead digestion protocol on next page, 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.

Notes: 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 protein removal.

Spin-Filters (low protein binding, 0.45 µm filter element) can be purchased separately, please inquire.

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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:

Albumin & IgG Removal products:
https://www.biotechsupportgroup.com/Albumin-Removal-s/307.htm

Lipid Removal Reagent and Clarification products:
https://www.biotechsupportgroup.com/Lipid-Removal-s/316.htm

Hemoglobin Removal products:
https://www.biotechsupportgroup.com/Hemoglobin-Removal-s/312.htm

CONTACT US

We welcome your questions and comments regarding our products.

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