NuGel™ Phenyl Boronic Acid

Polymer Coated Silica Affinity Matrices For Glycoprotein Purification.

Special Features of NuGel™:

- Non-specific sites are virtually eliminated by a polymer coating
- Stable across a wide pH range 2 - 10
- 1000Å, 50µm Silica suitable for LC and batch processes

Special Features of Phenyl Boronic Acid Ligand:

- Binds ligands containing 1,2 cis-diol groups of glycoproteins.
- pH stable from 2 to 10.

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.

Product Name	Application	Size	Column Volume approximately	Item No.
NuGel™ Phenyl Boronic Acid	Cis-diols, Glycoproteins	5 Grams	10 ml	NPBA-05
NuGel™ Phenyl Boronic Acid	Cis-diols, Glycoproteins	10 Grams	20 ml	NPBA-10

* Kilogram quantities and other particle sizes and porosity of NuGel™ are also available upon request. e.g.: 10 microns and 30 microns are available.

NuGel™ Phenyl Boronic Acid Characteristics

NuGel™ Phenyl Boronic Acid is a derivative of NuGel™ poly-epoxy affinity support. This affinity support contains phenyl boronic groups at the end of hydrophilic spacer arms and is used to bind ligands containing cis-diols, glycoproteins.

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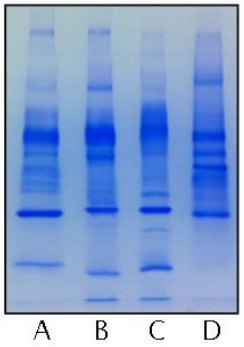
Characteristics Of The Matrix	
Operating Modes	Batch mode or in column mode
Space Arm	Polymerized hydrophilic carbon chain
Porosity	1000Å
Average Particle Size	50µm

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



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

NuGel™ Phenyl Boronic Acid Protocol

Recommended Items	Content
Binding Buffers Recommended	0.05M HEPES or 0.05M Taurine at pH 8.5
Wash Buffers Recommended	0.05M HEPES or 0.05M Taurine at pH 8.5
Elution Buffers Recommended	Binding Buffer containing 100mm Sorbitol at pH 8.5

Glycoprotein Affinity Purification

1) Protocol – Based on processing 50 ul Serum or 1-2 mg total protein.
2) Weigh out 50 mg of **NuGel™ Glycoprotein matrix, NPBA.**

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3) Add 500 µl of Recommended Binding Buffer (see above table) to the matrix. Vortex for 5 minutes and centrifuge for 5 minutes at 2,000-3,000 g. Discard the filtrate (Flow-Through).

4) Condition the sample by adding 450 µl of Recommended Binding Buffer (see above table) to 50 µl of sample. Vortex for 10 minutes and then centrifuge for 5 minutes at 2,000-3,000 g and discard the filtrate (Flow-Through).

5) Add 350 µl of Recommended Wash Buffer (see above table, same as Binding Buffer). Vortex for 5 minutes then centrifuge for 5 minutes at 2,000-3,000 g. Repeat this step 2 additional 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.

6) Add 300 µl of Recommended Elution Buffer (see above table). Vortex for 10 minutes and centrifuge for 5 minutes at 2,000-3,000 g. 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 sample volumes. The surface amount can be adjusted to accommodate more or less glycoprotein binding.
- Glycoprotein binding is approximately 5-10 mg/gm of NPBA matrix.

Suggested On-Bead Digestion Protocol

- After the final wash steps from step 5, 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.

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RELATED SAMPLE PREP & ENRICHMENT 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

CONTACT US

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