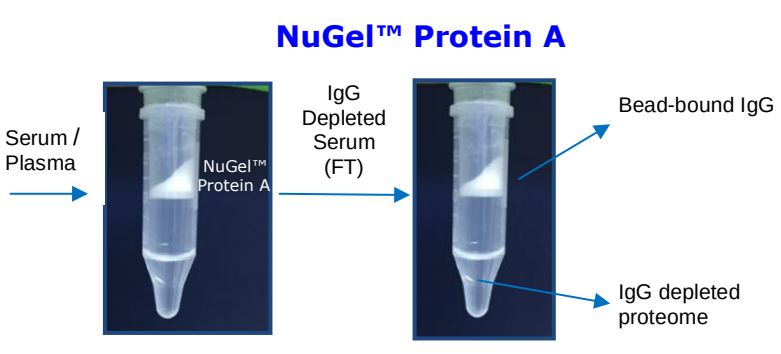
BIOTECH SUPPORT GROUP

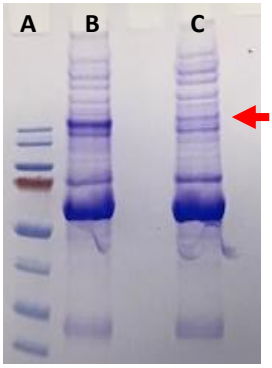
NuGel™ Protein A Kit

IgG Depletion From Serum/Plasma for Proteomics

- IgG removal >90% (70-80% of total Immunoglobulins removed) from serum or cell culture
- Supplied as dry stable bead format
- Seamless and simple < 1 hour protocol
- Disposable, cost-effective, no column regeneration or cross-contamination
- Works for most species tested including human, sheep, rat, mouse, bovine
- Suitable for LC-MS, 1 and 2D Gels, ELISAs, Enzyme and other Functional Assays
- IgG recoverable with acidic eluent (optional)
- Compatible with automation/high-throughput DPX Technologies XTR tip format

Typical Proteomics Performance	NuGel™ Protein A
Serum Sample Volume	25 – 50 µl
IgG Removal (most species)	>90%
IgM Removal (may vary with species)	30-50%
IgA Removal (may vary with species)	20-30%
IgD Removal (may vary with species)	20-30%
IgE Removal (may vary with species)	>90%
Total Immunoglobulin Removal (most species)	70-80%
Recoverable Protein Mass	1 - 2 mg (IgG depleted)
LC-MS/MS unique proteins (>2 Sp. Ct) (single 2 hr gradient)	200-300
For targeted proteomics, please contact technical services, as we have a knowledgebase of over 1000 serum proteins to help select the best method(s) for particular protein(s).	





SDS-PAGE Human Serum
20µg of protein loaded onto 10% bis tris gel

IgG depleted region

A: Markers
B: Neat Serum
C: After NuGel™ Protein A

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Product	Size	# Serum Preps	Item No.
NuGel™ Protein A Kit	20 preps	20, 25µl Serum samples	NP-20
NuGel™ Protein A Kit	100 preps	100, 25µl Serum samples	NP-100

Items included in NuGel™ Protein A kit

Items	Item No. NP-20	Item No. NP-100	Reagents
NuGel™ Protein A Beads (NP)	1.2 g	6 g	Supplied
Binding Buffer BB1 (0.05M K_2HPO_4 Dibasic, pH 7.5)	20 ml	100 ml	Supplied
Spin-Filter/tube assemblies	20	100	Supplied
Elution or Digest Buffers			Not Supplied (recommendations are noted in the protocol

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

NuGel™ Protein A – IgG depletion Protocol– Based on processing 25µl Serum; maximum 50 µl can be used

For best results – the serum should be clear and free of colloidal material. We recommend first filtering through a 0.45 µm syringe-type filter before beginning the prep.

1. Weigh out 60 mg of **NuGel™ Protein A** beads into the supplied microfuge Spin-filters.
2. Add 400 µl of **Buffer BB1** to condition the **NuGel™ Protein A beads**. Vortex for 3 min. Centrifuge for 3 minutes at room temperature at 5000 rpm. Discard the filtrate.
3. Add 100-250 µl of **Buffer BB1** to 25-50 µl of serum to **NuGel™ Protein A beads**. Vortex for 10 minutes. Centrifuge for 4 minutes at 10,000 rpm.
4. For wash, add 200 µl of the **Buffer BB1** to beads. Vortex for 10 minutes.
5. Centrifuge for 4 minutes at 10,000 rpm, 200-450 µl **filtrate contains serum proteins depleted of IgGs. Beads contain bound Immunoglobulin (mostly IgG) fraction and associated antigens. For LC-MS proteomics, bound fraction can be eluted (a) or digested on-bead (b).**

   a. To recover bound IgG fraction, use 250 µl of acidic buffers such as 0.1 M sodium citrate, pH 3.3, or 0.1 M Glycine pH 3-4.

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b. On-Bead Digest – BASP™ (Bead-assisted Sample Prep)

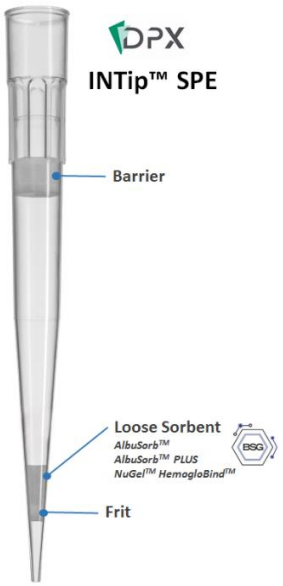
1. Using 0.05M HEPES pH 7 buffer, prepare to 10mM of DTT concentration, and add 100 µl to the beads and vortex for 10 minutes and incubate for 30 minutes at 60C.
2. Cool the samples to RT, add suitable volume of Iodoacetamide to 20mM and incubate in the dark for 45 minutes
3. Centrifuge at 10,000rpm for 4 minutes and discard the filtrate.
4. Rinse the bottoms of the spin-X tubes with 500 µl of 50% ACN, 0.05M HEPES pH 7 buffer twice, to remove any traces of the filtrate.
5. Add 8 µg trypsin in 200 µl 0.05M HEPES pH 7 buffer to the beads and keep at 37°C for a minimum 4 hours to maximum overnight. Overnight is recommended to start with. In select targeted circumstances, 2 hours may be sufficient.
6. Centrifuge at 10,000rpm for 4 minutes and collect the filtrate.
7. Add 150 µl of 10% formic acid to extract further peptides, vortex for 10 minutes and centrifuge at 10,000rpm for 4 minutes. Combine the filtrate (Total 350 µl).
8. Dry the unused filtrate and store at -80°C. The sample is ready for LC-MS.

Compatible with the high-throughput XTR tip format

The XTR tip format improves ease of use and scalability to process multiple samples in parallel, utilizing 96-well plates and automated liquid handlers. INTip™ SPE formats have been proven to be compatible with most automation platforms, i.e., Integra, Hamilton, etc. The **NuGel™ Protein A** beads are loosely contained inside the XTR tips for a dispersive functionality that maximizes depletion efficacy.

A poster report is downloadable at:
https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/ASMSBSGDPXPoster.pdf

Please inquire for price and availability.



CONTACT US

We welcome your questions and comments regarding our products.

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