Protein A Mag Sepharose™
Protein G Mag Sepharose
NHS Mag Sepharose

Protein A Mag Sepharose, Protein G Mag Sepharose, and NHS Mag Sepharose are magnetic beads designed to simplify enrichment of target proteins by immunoprecipitation or pull-down applications (Fig 1). Antibodies or other proteins immobilized to Mag Sepharose beads are used for capture of target proteins after which collection of the beads is achieved using a magnetic device.

Key benefits of Mag Sepharose beads are:

- Visible and dense, easy to spot and collect bound target protein
- Non-adherent beads eliminate smearing effects and aggregate formation. Can be used without detergents
- Simple capture of target protein in small or large sample volumes (low microliter to high milliliter scale)
- Optimized capacity for enrichment/immunoprecipitation of target proteins—only low amounts of antibody needed
- Flexible protocols with elution conditions optimized for both electrophoresis and MS analysis

The beads are each available in two pack sizes: 1 × 500 µl slurry suitable for 20 samples and 4 × 500 µl suitable for 80 samples. Together with MagRack 6, a separation tool for handling the beads in microcentrifuge tubes, up to six samples can be processed in parallel.

Fig 1. Protein A Mag Sepharose, Protein G Mag Sepharose, and NHS Mag Sepharose are designed for efficient small-scale protein enrichment from clarified cell lysates and biological fluids.

Reliable capture of target protein

Protein A Mag Sepharose and Protein G Mag Sepharose are affinity chromatography media (resins) with protein A or protein G ligands, both of which have high affinity for monoclonal or polyclonal antibodies. The antibodies immobilized to the protein A or protein G ligands of the Mag Sepharose media are used for capture of target proteins by immunoprecipitation.

NHS Mag Sepharose is designed for covalent coupling of any protein or biomolecule containing a free amine group and is used for various types of pull-down experiments.

Simplified handling

The magnetic bead format has excellent properties for small-scale experiments. The high density of the beads allows rapid capture by magnetic devices while the visibility of the beads ensures reliable collection of all target protein bound in the immunoprecipitation/pull-down application. This allows concentration of low-abundant target protein samples from milliliter down to microliter scale. Starting from 25 ml sample, it is possible to obtain the enriched products in volumes as small as 2.5 µl. The characteristics of the media are summarized in Table 1.

All products are provided with protocols optimized for sample preparation for downstream analysis, such as MALDI-ToF and LC-MS.

MagRack 6 enable preparation of up to six samples captured in 1.5 ml microcentrifuge tubes. When the tubes are placed in the rack, the magnetic beads are attracted to the magnet within a few seconds. This allows easy removal of the supernatant whereas the magnetic beads are left in the tube. For larger sample volumes, a 50 ml plastic tube can be used when binding the target protein. A magnetic pickpen is recommended for the transfer of the magnetic beads to a smaller tube after initial capture of the target protein. An alternative is to spin down the beads by using a swing-out centrifuge.

For increased convenience, Protein A/G SpinTrap Buffer Kit and NHS SpinTrap Buffer Kit are available. These buffer kits eliminate time-consuming buffer preparations and thus promote fast and reproducible protein enrichment.

Table 1. Characteristics of Protein A Mag Sepharose, Protein G Mag Sepharose, and NHS Mag Sepharose

Protein A Mag Sepharose	
Matrix	Paramagnetic, spherical, highly cross-linked agarose particles
Ligand	Native protein A
Binding capacity	8–17 mg human IgG/ml gel
Particle size	37–100 µm
Working temperature	Room temperature
Storage solution	20% ethanol
Storage temperature	4°C to 8°C
Protein G Mag Sepharose	
Matrix	Paramagnetic, spherical, highly cross-linked agarose particles
Ligand	Protein G
Binding capacity	13–22 mg human IgG/ml gel
Particle size	37–100 µm
Working temperature	Room temperature
Storage solution	20% ethanol
Storage temperature	4°C to 8°C
NHS Mag Sepharose	
Matrix	Paramagnetic, spherical, highly cross-linked agarose particles
Ligand	N-hydroxysuccinimide
Ligand density	8–14 µmol/ml gel
Particle size	37–100 µm
Working temperature	Room temperature
Storage solution	2-propanol
Storage temperature	4°C to 8°C

Flexible protocol

For Protein A Mag Sepharose or Protein G Mag Sepharose, there are two alternative protocols for protein enrichment: (1) the cross-link protocol where the target protein is eluted whereas the antibodies remain covalently bound to the matrix by using a cross-linking agent, and (2) the classic protocol, where the target protein is eluted together with the antibodies.

NHS Mag Sepharose is pre-activated, and proteins, antibodies from various species, and aptamers can be covalently coupled via primary amino groups. In a second step, target biomolecules are affinity-captured and eluted as in the cross-link protocol.

The optimal parameters for protein enrichment are dependent on the specific combination of biomolecules present in the sample. Optimization of buffers may therefore be required for each specific combination in order to obtain the best result. The product instructions list recommended buffers.

Reproducible protein enrichment: a benchmark analysis

To demonstrate efficiency and reproducibility, Protein A Mag Sepharose was evaluated in GE Healthcare's laboratories by running nine replicates. As a comparison, a parallel experiment was set up with Dynabeads™ Protein A (Invitrogen Corporation).

Human transferrin labeled with Cy™5 minimal dye, was enriched from a background of non-labeled *E. coli* proteins using a polyclonal rabbit anti-human transferrin antibody. For both media, the cross-link protocol was followed, and each medium was treated according to the instructions delivered with the corresponding product (Table 2). Eluted fractions were analyzed with SDS-PAGE (ExcelGel™), post-stained with Deep Purple™ total protein stain, and scanned in Ettan™ DIGE Imager.

Protein A Mag Sepharose displayed a significantly higher recovery compared with Dynabeads Protein A, a recovery average of 53% and 20%, respectively (Fig 2). The purity was more or less the same with a purity average of 52% for Protein A Mag Sepharose and 50% for Dynabeads Protein A respectively.

Table 2. Experimental conditions

	Protein A Mag Sepharose	Dynabeads Protein A
Separation medium	25 µl gel slurry, Protein A Mag Sepharose	50 µl gel slurry, Dynabeads Protein A
Sample	5 mg/ml *E. coli* protein containing 7.5 µg/ml human transferrin	5 mg/ml *E. coli* protein containing 7.5 µg/ml human transferrin
Sample volume	200 µl	200 µl
Antibodies	Polyclonal rabbit anti-human transferrin	Polyclonal rabbit anti-human transferrin
Binding buffer	Tris buffered saline (TBS); 50 mM Tris, 150 mM NaCl, pH 7.5	0.1 M sodium phosphate, 0.01% Tween™ 20, pH 8.2
Wash buffer	TBS, 2 M urea, pH 7.5	Phosphate buffered saline (PBS)
Elution buffer	2 × 50 µl 0.1 M glycine/HCl, 2 M urea, pH 2.9	2 × 50 µl 0.1 M glycine/HCl, pH 2.9

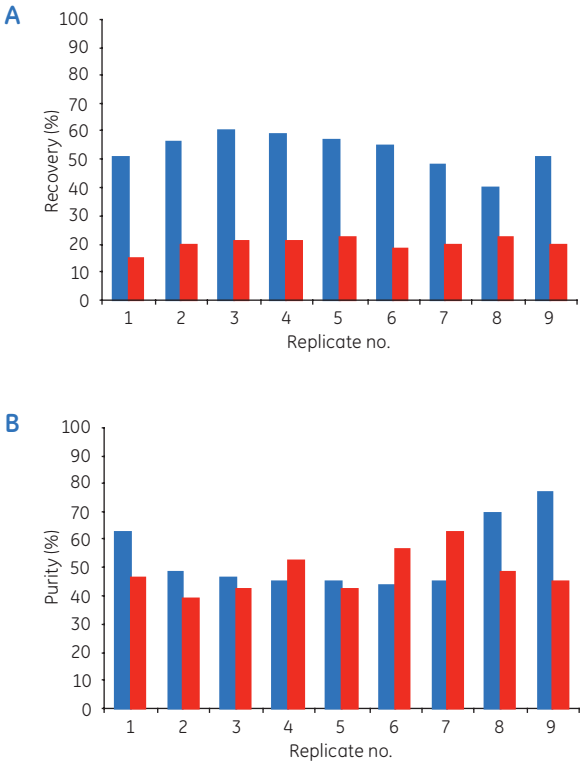


Fig 2. Comparison between Protein A Mag Sepharose (blue) and Dynabeads Protein A (red) with respect to **(A)** recovery, and **(B)** purity of human transferrin.

Finding low-abundant Tyr-phosphorylated proteins in complex mixtures

Proteins active in signaling pathways are normally not detectable with SDS-PAGE or MS analysis without sample preparation due to low abundance.

Protein G Mag Sepharose medium with immobilized anti-pTyr antibody was used to enrich and concentrate tyrosine-phosphorylated proteins and allow elution in volumes suitable for MS analysis.

CHO cells (7×10^7) were pretreated with 100 µM pervanadate for 3 h before harvest and lysis. The lysate, including protease and phosphatase inhibitors, was clarified by centrifugation and diluted two-fold before incubation with anti-pTyr antibodies immobilized on Protein G Mag Sepharose for 60 min at room temperature (end-over-end). Tyrosine-phosphorylated proteins were eluted with 2 × 100 µl of 100 mM phenylphosphate. Trypsin digestion was performed before LC-MS/MS analysis. Untreated cells were prepared as control.

MS identification gave 76 hits of potential tyrosine-phosphorylated proteins of which 54 were exclusively found in the pervanadate-treated cells (data not shown). Most of these proteins belonged to focal adhesion-related pathways. Untreated cells gave only 22 hits, mainly high-abundant enzymes and ribosomal proteins.

In combination with anti-pTyr antibodies, Protein G Mag Sepharose is a powerful tool for capturing low-abundant proteins involved in different signaling pathways from large amount of starting samples.

Enrichment of plasminogen from human plasma

NHS Mag Sepharose is the obvious first choice for immunoprecipitation in plasma samples since endogenous immunoglobulins may bind to remaining free ligands of Protein A Mag Sepharose or Protein G Mag Sepharose. Furthermore, some antibodies, for example mouse IgG1, are known to bind poorly to Protein A and Protein G under normal conditions.

Human plasma contains a vast number of proteins and can be difficult to work with due to the great range of protein concentrations. This immunoprecipitation experiment demonstrates an efficient enrichment of plasminogen from plasma sample using a mouse monoclonal antibody coupled to NHS Mag Sepharose.

Plasminogen was enriched from human plasma using an anti-plasminogen mouse IgG1 monoclonal antibody covalently coupled to NHS Mag Sepharose. In a parallel experiment, the same antibody was captured on Protein A Mag Sepharose. The antibodies were first mixed with 1.2 M KH_2PO_4 in order to increase the affinity of the mouse IgG1 antibody to the protein A ligand, and the antibodies were cross-linked.

To remove endogenous IgG, the sample was clarifiied on a HiTrap™ Protein A HP column. Fractions from both enrichment experiments were analyzed on SDS-PAGE (Fig 3). The plasminogen was further identified by LC-MS/MS.

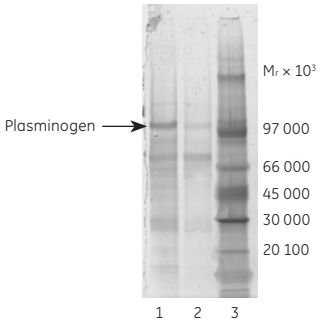
Both experimental setups ensured a high level of enrichment of the target protein. The poor affinity of protein A for a monoclonal mouse IgG1 can be overcome by slight modification of the protocol.

A. NHS Mag Sepharose

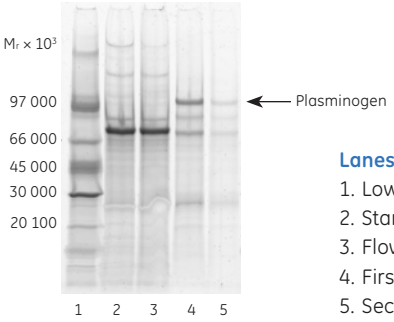
Affinity medium	25 µl NHS Mag Sepharose
Sample	Human plasma
Sample volume	6 ml
Antibodies	Anti-plasminogen mouse monoclonal IgG1
Coupling buffer	0.15 M triethanolamine, 0.5 M NaCl, pH 8.3
Binding buffer	Tris buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5)
Wash buffer	TBS, 2 M urea, pH 7.5
Elution buffer	2 × 50 µl 0.1 M glycine/HCl, 2 M urea, pH 2.9

B. Protein A Mag Sepharose

Affinity medium	25 µl Protein A Mag Sepharose
Sample	Human plasma
Sample volume	10 ml
Antibodies	Anti-plasminogen mouse monoclonal IgG1
Binding buffer	1.2 M KH_2PO_4, pH 9 (increases the affinity for mouse IgG1 to Protein A)
Wash buffer	TBS, 2 M urea, pH 7.5
Elution buffer	2 × 50 µl 0.1 M glycine/HCl, 2 M urea, pH 2.9



Lanes
1. First elution
2. Second elution
3. Low molecular weight markers



Lanes
1. Low molecular weight markers
2. Start material (diluted 1:8)
3. Flowthrough (diluted 1:8)
4. First elution
5. Second elution

Fig 3. Enrichment of plasminogen from human plasma. **(A)** SDS-PAGE results of NHS Mag Sepharose. **(B)** SDS-PAGE results of Protein A Mag Sepharose. The gels were post-stained with Deep Purple Total Protein Stain and scanned using Ettan DIGE Imager. The arrows indicate the position of the plasminogen.

Ordering information

Product	Quantity	Code no.
Protein A Mag Sepharose	1 × 500 µl	28-9440-06
Protein A Mag Sepharose	4 × 500 µl	28-9513-78
Protein G Mag Sepharose	1 × 500 µl	28-9440-08
Protein G Mag Sepharose	4 × 500 µl	28-9513-79
NHS Mag Sepharose	1 × 500 µl	28-9440-09
NHS Mag Sepharose	4 × 500 µl	28-9513-80

Related products	Quantity	Code no.
MagRack 6	1	28-9489-64
TiO_2 Mag Sepharose	1 × 500 µl	28-9440-10
TiO_2 Mag Sepharose	4 × 500 µl	28-9513-77
Protein A/G SpinTrap Buffer Kit	1	28-9135-67
NHS HP SpinTrap Buffer Kit	1	28-9135-69
Mammalian Protein Extraction Buffer	1 × 500 ml	28-9412-79
Protease Inhibitor Mix	1 ml	80-6501-23

For local office contact information, visit
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