GE Healthcare

Protein A Mag Sepharose
Protein G Mag Sepharose

Protein A Mag Sepharose™ and Protein G Mag Sepharose are available in the following formats (Instructions for use included in all formats):

- 1 × 500 µl Protein A coupled magnetic Sepharose 4 Fast Flow
- 4 × 500 µl Protein A coupled magnetic Sepharose 4 Fast Flow
- 1 × 500 µl Protein G coupled magnetic Sepharose 4 Fast Flow
- 4 × 500 µl Protein G coupled magnetic Sepharose 4 Fast Flow

Purpose

Protein A Mag Sepharose and Protein G Sepharose products are magnetic beads designed for coupling of antibodies enabling enrichment of target protein for further downstream analyses such as mass spectrometry (MS and LC-MS) and electrophoresis techniques.

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1 Principle

Protein A Mag Sepharose and Protein G Mag Sepharose are affinity chromatography media with high affinity for antibodies from various species, which makes them useful for enrichment of target protein using immunoprecipitation technology. The products are magnetic beads based on Sepharose 4 Fast Flow.

Mag Sepharose products are used together with Eppendorf microcentrifuge tubes and a magnetic rack, for example MagRack 6 (see Section 6. Ordering Information). The magnetic beads are easily separated from the liquid phase during the different steps of the immunoprecipitation protocol.

Protocols

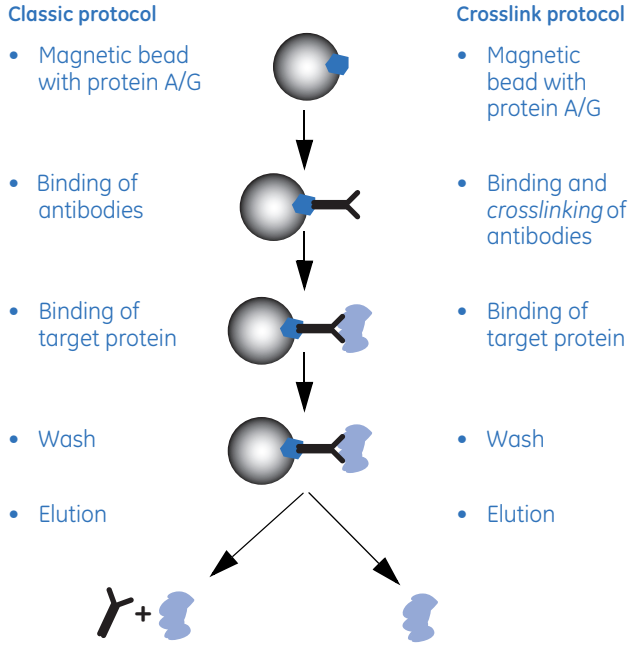
There are two protocols for protein enrichment using Protein A Mag Sepharose or G Mag Sepharose:

- Crosslink protocol

  The target protein is eluted whereas the antibodies remain covalently bound to the matrix by using a crosslinking agent.

- Classic protocol

  The target protein is eluted together with the antibodies.



2 Advice on handling

General handling

Dispensing the medium slurry

- Prior to dispensing the medium slurry, make sure it is homogeneous by vortexing or by repeated manual inversion of the vial.

  When the medium slurry is resuspended, pipette *immediately* the required amount of medium slurry into the Eppendorf tube.

  Repeat the re-suspension step between every pipetting from the medium slurry vial.
- Use 1.5 ml Eppendorf tubes.

Handling of liquid

- Use the magnetic rack with the magnet in place for each liquid removal step.
- Before application of liquid, wash buffer, elution buffer etc., remove the magnet from the magnetic rack.

  After addition of liquid, allow re-suspension of the beads by vortex or manual inversion of the Eppendorf tube.

  When processing multiple samples, manual inversion of the magnetic rack is recommended.

Incubation steps

- During incubation steps, make sure the gel beads are well resuspended and kept in solution by end-over-end mixing or by using a benchtop shaker suitable for 1.5 ml Eppendorf tubes.
- Incubation steps take generally place at room temperature. However, incubation could take place at +4°C over night if this is the recommended storage condition for the specific sample.
- If needed, add a centrifugation step (microcentrifuge) to remove liquid from the lid, especially before the elution step.

Sample pretreatment

- Clarifying of sample might be needed before applying it to the beads.
- To prevent target protein degradation, inhibition of protease activity may be required, a Protease Inhibitor Mix is available, see Section 6.

Choice of protocol

Crosslink protocol

- If the desired protein/antigen has similar molecular weight as the heavy or light chain of the antibody which causes problem with co-migration in SDS-PAGE analysis.
- If the antibody interferes with downstream analyses.

Classic protocol

- If the antibody does not interfere with the downstream analyses and does not shadow the protein of interest in for example SDS-PAGE analysis.
- If faster processing is desired.

Tips and Hints

- In case of larger volume of starting material (> 1.5 ml), a 50 ml plastic tube could be used when binding the target protein.
- To recover the magnetic beads after incubation, a magnetic pickpen could be used for transferring the beads to an Eppendorf tube. Another alternative is to spin down the beads by using a swing-out centrifuge.
- It could be advisable to transfer the magnetic bead solution to a fresh Eppendorf tube during the last wash buffer step. This action prevents potential elution of proteins non-specifically bound to the plastic material in the Eppendorf tube.
- After elution, place the fractions in the freeze or add sample buffer if SDS-PAGE is to be performed to prevent sample degradation.

3 Optimization of parameters

The optimal parameters for protein enrichment are dependent on the specific combination of biomolecules used. Optimization may be required for each specific combination to obtain the best result.

Examples of parameters which may require optimization are:

- Amount of beads
  - a recommended starting volume could be 5 µl magnetic beads (25 µl medium slurry)
- Amount of antibodies
- Amount of protein (antigen) to be enriched
- Incubation times
- Choice of buffers
- Number of washes

Choice of buffers

It is recommended to use the listed buffers for the indicated type of protocol.

A Protein A/G Buffer Kit is available as an accessory for increased convenience, see Section 6.

Recommended buffers Crosslink protocol

Buffer	Composition
Binding buffer	• TBS (50 mM tris, 150 mM NaCl pH 7.5)
Wash buffer	• TBS with 2 M urea. pH 7.5
Elution buffer	• 0.1 M glycine-HCl with 2 M urea, pH 2.9
Crosslink solutions	• 200 mM triethanolamine pH 8.9 (crosslink solution A) • 50 mM DMP (dimethyl pimelimidate dihydrochloride) in 200 mM triethanolamine pH 8.9 • 100 mM ethanolamine, pH 8.9 (crosslink solution B)

Recommended buffers Classic protocol

Buffer	Composition
Binding buffer	• TBS (50 mM tris, 150 mM NaCl pH 7.5)
Wash buffer	• TBS (50 mM tris, 150 mM NaCl pH 7.5)
Elution buffer	• 2.5% acetic acid (HAc)

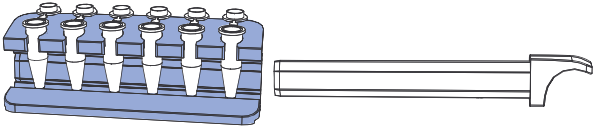
Alternative buffers

Buffer	Composition
Binding buffer	• 1.2 M KH_2PO_4 pH 9.0 (increases the affinity for mouse IgG 1 to Protein A)
Wash buffer	• TBS, pH 7.5 (mild wash) • TBS with 1% octylglucoside. pH 7.5 • 0.1 M triethanolamine, 0.5 M NaCl pH 9.0
Elution buffer	• 0.1 M glycine-HCl, pH 2.5 to 3.1 • 0.1 M citric acid, pH 2.5 to 3.1 • 2.5% HAc • 2% SDS • 0.1 M ammonium hydroxide, pH 10.0 to 11.0 • 100 mM phenyl phosphate (suitable for elution of phosphoproteins)

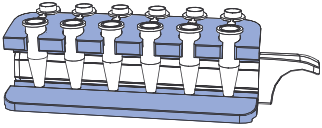
4 Protocols

General magnetic separation step

1 *Remove* the magnet before *adding* liquid.



2 *Insert* the magnet before *removing* liquid.



Crosslink protocol

Preparations

- Prepare the required amount of washing solution by adding urea to a portion of binding buffer to a final concentration of 2 M.
- Prepare the required amount of elution buffer by adding urea to a portion of elution buffer to a final concentration of 2 M.
- Prepare the antibody solution by diluting the required amount of antibody in binding buffer, minimum total volume 50 µl, to achieve a proper mixing during the immobilization step.

1 Magnetic bead preparation

A Dispense the required amount of magnetic beads into the Eppendorf tube.

B Place the Eppendorf tube in the magnetic rack and remove the storage solution.

2 Equilibration

A Add 500 µl binding buffer.

B Resuspend the medium.

C Remove the liquid.

3 Binding of antibody

A Immediately after equilibration, add the antibody solution.

B Resuspend the medium and let incubate with slow end-over -end mixing for at least 15 minutes.

C Remove the liquid.

4 Washing

A Add 500 µl binding buffer.

B Remove the liquid.

5 Buffer change

A Add 500 µl triethanolamine (crosslink solution A)

B Remove the liquid.

6 Crosslink

A Add 500 µl triethanolamine (crosslink solution A) with 50 mM DMP.

B Fully resuspend the medium by manual inversion and incubate with slow end-over-end mixing for 15 to 60 minutes.

C Remove the liquid.

7 Wash

A Add 500 µl triethanolamine (crosslink solution A).

B Remove the liquid.

8 Blocking

A Add 500 µl ethanolamine (crosslink solution B).

B Fully resuspend the medium by manual inversion and incubate with slow end-over-end mixing for 15 minutes.

C Remove the liquid.

9 Removal of non-bound antibody

A Add 500 µl elution buffer.

B Remove the liquid.

10 Wash (perform this step 2 times totally)

A Add 500 µl binding buffer.

B Remove the liquid.

11 Binding of target protein

A Add sample (diluted in e.g. binding buffer).

B Incubate with slow end-over-end mixing for 10 to 60 minutes.

C Remove and *collect* the non-bound fraction.

12 Wash (perform this step 3 times totally)

A Add 500 µl wash buffer.

B Remove the liquid.
Optional: Collect the washes in case trouble shooting is required.

13 Elution (perform this step 2 times totally)

A Add 10 volumes of elution buffer compared to the magnetic bead volume. For example 50 µl buffer to 5 µl magnetic beads (25 µl medium slurry).

B Fully resuspend the medium and let incubate for at least 2 minutes.

C Remove and *collect* the elution fraction.

Classic Protocol

Preparations

Prepare the antibody solution by diluting the required amount of antibody in binding buffer before running the protocol.

1 Magnetic bead preparation

A Dispense the required amount of magnetic beads into the Eppendorf tube.

B Place the Eppendorf tube in the magnetic rack and remove the storage solution.

2 Equilibration

A Add 500 µl binding buffer.

B Resuspend the medium.

C Remove the liquid.

3 Binding of antibody

A Immediately after equilibration, add the antibody solution.

B Resuspend the medium and let incubate with slow end-over-end mixing for at least 15 minutes.

C Remove the liquid.

4 Washing

A Add 500 µl binding buffer.

B Remove the liquid.

5 Binding of target protein

A Add sample (diluted in e.g. binding buffer).

B Incubate with slow end-over-end mixing for 10 to 60 minutes.

C Remove and *collect* the non-bound fraction.

6 Wash (perform this step 3 times totally)

A Add 500 µl wash buffer.

B Remove the liquid.
Optional: Collect the washes in case troubleshooting is anticipated.

7 Elution (perform this step 2 times totally)

A Add 10 volumes of elution buffer compared to the magnetic bead volume. For example 50 µl buffer to 5 µl magnetic beads (25 µl medium slurry).

B Fully resuspend the medium and let incubate for at least 2 minutes.

C Remove and *collect* the elution fraction.

5 Characteristics

Table 1. Protein A Mag Sepharose.

Matrix	Highly crosslinked spherical agarose (Sepharose 4 Fast Flow) including magnetite
Medium	Protein A coupled NHS activated Mag Sepharose
Ligand	Protein A
Binding capacity	>8 mg human IgG/ml gel
Particle size	37 to 100 µm
Working temperature	Room temperature
Storage solution	20% ethanol, 20% medium slurry
Storage temperature	+4°C to +8°C

Table 2. Protein G Mag Sepharose.

Matrix	Highly crosslinked spherical agarose (Sepharose 4 Fast Flow) including magnetite
Medium	Protein G coupled NHS activated Mag Sepharose
Ligand	Protein G
Binding capacity	>13 mg human IgG/ml gel
Particle size	37 to 100 µm
Working temperature	Room temperature
Storage solution	20% ethanol, 20% medium slurry
Storage temperature	+4°C to +8°C

6 Ordering Information

Products	Quantity	Code No.
Protein A Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-06
Protein A Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-78
Protein G Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-08
Protein G Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-79

Related products	Quantity	Code No.
NHS Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-09
NHS Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-80
MagRack 6	1	28-9489-64
Protein A/G HP SpinTrap™ Buffer Kit	1	28-9135-67
TiO_2 Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-10
TiO_2 Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-77
Protease Inhibitor Mix	1 ml	80-6501-23

Literature	Code No.
Data file: NHS Mag Sepharose Protein A Mag Sepharose Protein G Mag Sepharose	28-95-39-39

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/sampleprep

GE Healthcare Europe GmbH
Munzinger Strasse 5,
D-79111 Freiburg,
Germany

GE Healthcare UK Ltd
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue
P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg.
3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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