NHS Mag Sepharose

NHS Mag Sepharose™ is available in the following formats (Instructions for use are included):

- 1 × 500 µl NHS coupled magnetic Sepharose 4 Fast Flow
- 4 × 500 µl NHS coupled magnetic Sepharose 4 Fast Flow

Purpose

NHS Mag Sepharose is magnetic beads designed for coupling of antibodies, aptamers and proteins through primary amino groups on the molecules to the NHS ligand on NHS Mag Sepharose.

This enables enables enrichment of target protein for further downstream analyses such as mass spectrometry (MS(/LC-MS) and electrophoresis techniques.

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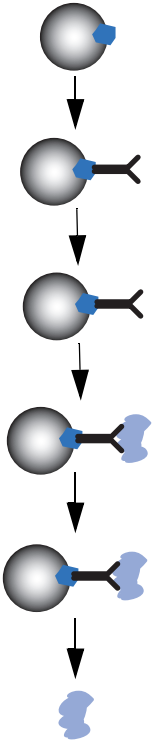
1 Principle

NHS Mag Sepharose is a N-hydroxysuccinimide activated Sepharose 4 Fast Flow, a medium to which molecules with primary amino groups bind covalently.

Proteins, antibodies and aptamers from various species can be coupled to NHS. Subsequently, proteins of interest are affinity captured and enriched using immunoprecipitation technology.

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.

Principle



- Magnetic bead with NHS
- Binding of antibodies
- Blocking of residual active groups
- Binding of target protein
- Wash
- Elution

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 resuspension 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 resuspension 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.

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

- Do not use an amine-containing buffer (e.g. Tris or glycine) for the antibody/protein that is to be coupled since the amines in the buffer will compete for the coupling sites.
- Remove potential amines before coupling for example by dialysis or buffer exchange with a desalting column.
- It is recommended to use the buffers listed below
- All buffers/solutions needed except HCl are also available in a buffer kit, *NHS HP SpinTrap™ Buffer Kit*, as an accessory for increased convenience, see Section 6.
- If optimization is required, alternative buffers are listed below.

Recommended buffers

Buffer	Composition
Equilibration buffer	• 1 mM HCl (ice cold)
Coupling buffers	• 0.15 M triethanolamine, 0.5 M NaCl, pH 8.3
	• 0.2 M $NaHCO_3$, 0.5 M NaCl, pH 8.3
Blocking buffer A	• 0.5 M ethanolamine, 0.5 M NaCl, pH 8.3
Blocking buffer B	• 0.1 M Na-acetate, 0.5 M NaCl, pH 4.0
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, 2 M urea, pH 2.9

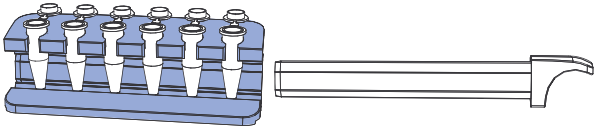
Alternative buffers

Buffer	Composition
Blocking buffers	• 50 mM Tris-HCl, 1 M NaCl, pH 8.0
	• 50 mM glycine-HCl, 1 M NaCl, pH 3.0
Elution buffers	• 0.1 M glycine-HCl, pH 2.5 to 3.1
	• 0.1 M citric acid, pH 2.5 to 3.1
	• 2.5% acetic acid
	• 2% SDS
	• 0.1 M ammonium hydroxide, pH 10.0 to 11.0

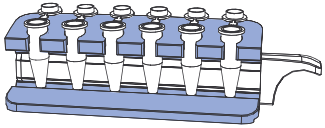
4 Protocol

General magnetic separation step

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



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



Preparations

- Prepare the antibody solution by dilution in coupling buffer and keep it on ice.
  Minimum total volume is 50 µl to achieve a proper mixing during the immobilization step.
- Prepare 1 mM HCl and make sure it is ice-cold before use
- Optional: Prepare the required amount of washing solution by adding urea to a portion of binding buffer to a final concentration of 2 M.
- Optional: Prepare the required amount of elution buffer by adding urea to a portion of elution buffer to a final concentration of 2 M

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 ice cold equilibration 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 Blocking of residual active groups

A Add 500 µl blocking buffer A and remove the liquid.

B Add 500 µl blocking buffer B and remove the liquid.

C Add 500 µl blocking buffer A.

D Incubate for 15 minutes with slow end-over-end mixing.

E Remove the liquid.

F Add 500 µl blocking buffer B and remove the liquid.

G Add 500 µl blocking buffer A and remove the liquid.

H Add 500 µl blocking buffer B and remove the liquid.

5 Equilibration for binding

A Add 500 µl binding buffer.

B Remove the liquid.

6 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.

7 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 anticipated.

8 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

Matrix	Highly crosslinked spherical agarose (Sepharose 4 Fast Flow) including magnetite
Medium	NHS activated Mag Sepharose
Ligand	N-hydroxysuccimide
Binding capacity	8 to 14 µmol/ml gel
Particle size	37 to 100 µm
Working temperature	Room temperature
Storage solution	2-propanol, 20% medium slurry
Storage temperature	+4°C to +8°C

6 Ordering Information

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

Related 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
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
MagRack 6	1	28-9489-64
NHS HP SpinTrap Buffer Kit	1	28-9135-69
Protease Inhibitor Mix	1 ml	80-6501-23

Literature	Code No.
Data file: NHS Mag Sepharose Protein A Mag Sepharose Protein G Mag Sepharose	28-9539-39

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