GE Healthcare
Life Sciences

Streptavidin Mag Sepharose™

Streptavidin Mag Sepharose is available in the following pack sizes (instructions for use are included in both the packs):

- Streptavidin Mag Sepharose, 10% medium slurry, 2 × 1 ml
- Streptavidin Mag Sepharose, 10% medium slurry, 5 × 1 ml

Note: *1 ml medium slurry is sufficient for 20 reactions according to the recommended immunoprecipitation protocol (see Section 5).*
1 ml of 10% (v/v) medium slurry contains 100 µl of magnetic beads.

Purpose

Streptavidin Mag Sepharose products contain magnetic beads that enable strong binding of biotinylated biomolecules. Two protocols are included in this instruction; one for capturing and eluting biotinylated proteins and another for enrichment of proteins by immunoprecipitation.

Intended use

Streptavidin Mag Sepharose is intended for research use only, and should not be used in any clinical or *in vitro* procedures for diagnostic purposes.

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1 Principle

Streptavidin Mag Sepharose is an affinity chromatography medium with high affinity for biotinylated proteins and other biotinylated biomolecules. The medium consists of streptavidin ligand coupled to magnetite-containing Sepharose beads.

The binding of streptavidin to biotin is one of the strongest known non-covalent biological interactions. Hence, denaturating conditions are generally required for the efficient elution of biotinylated biomolecules. Alternatively, biotinylated biomolecules bound to Streptavidin Mag Sepharose can be used to capture interacting target substances such as proteins. Impurities are removed by washing, and the enriched target protein is eluted using less harsh elution conditions.

Streptavidin Mag Sepharose provides flexible enrichment of biomolecules. The enrichment process can be easily scaled just by varying the volume of the Mag Sepharose beads.

For simplified handling, Mag Sepharose products can be used together with a magnetic rack, for example, MagRack 6 for sample volumes up to 1.5 ml or MagRack Maxi for sample volumes up to 50 ml (see Section 2).

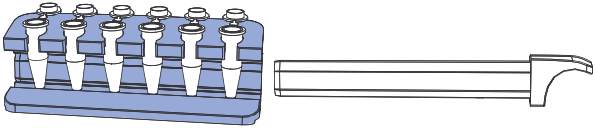
2 Advice on handling

Note: *Streptavidin Mag Sepharose is intended for single use only.*

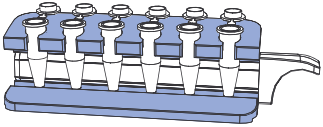
General magnetic separation

The protocols recommended in this instruction use 1.5 ml Eppendorf tubes and MagRack 6 (see Section 4 and Section 5).

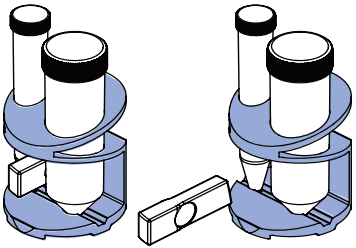
1 *Remove* the magnet before *adding* the liquid.



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



When using volumes above 1.5 ml, MagRack Maxi, designed for sample volumes up to 50 ml, is recommended.



For volumes more than 50 ml, the beads can be spun down using a swing-out centrifuge.

Dispensing the medium slurry

- Vortex the medium slurry to homogeneity before dispensing.
- Pipette out the required amount of medium slurry into the appropriate tube immediately after resuspension.
- Repeat the resuspension step between each pipetting step.

Handling the liquid

- Remove the magnet from the magnetic rack, before adding liquid to the tube.
- Resuspend the beads by vortexing or by inverting the tube manually, after adding the liquid.
- Use the magnetic rack with the magnet inserted for each liquid removal step.
- Remove the liquid from the lid of the vial using a pipette, if needed.

Incubating

- Resuspend the Mag Sepharose beads well in solution by mixing end-over-end or by using a benchtop shaker, during incubation.
- Longer incubation times might be necessary when purifying larger volumes of samples, samples having low affinity or samples with very low concentrations.

Note: *The samples can generally be incubated at room temperature. However, they can be incubated at +4°C also, if specifically recommended for the sample.*

3 Preparing buffers and samples

Recommended buffers

Use the listed recommended buffers. Use any of the alternative buffers for optimization.

Note: *Use high-purity water and chemicals for buffer preparation.*

Table 1. Recommended buffers for capturing and eluting biotinylated proteins

Buffer	Composition
Binding buffer	Tris-buffered saline (TBS) (50 mM Tris-HCl, 150 mM NaCl), pH 7.5
Washing buffer	TBS, 2 M urea, pH 7.5
Elution buffer	2% SDS

Table 2. Recommended buffers for immunoprecipitation

Buffer	Composition
Binding buffer	TBS, pH 7.5
Washing buffer	TBS, 2 M urea, pH 7.5
Elution buffer	0.1 M glycine-HCl, 2 M urea, pH 2.9

Table 3. Alternative buffers

Buffer	Composition
Washing buffer	• TBS, pH 7.5 (mild wash) • 0.1 M triethanolamine, 0.5 M NaCl, pH 9.0
Elution buffer	• 0.1 M glycine, pH 2.5 to pH 3.1 • 0.1 M citric acid, pH 2.5 to pH 3.1 • 2% SDS • 0.1 M ammonium hydroxide, pH 10.0 to pH 11.0

- Streptavidin HP Buffer kit is available for easy preparation of buffers (see Section 8).

- Purity of the sample might be improved by adding detergents or salts to the wash buffer.

Preparing the sample

- Adjust the sample to the composition and pH of the binding buffer. pH can be adjusted by either diluting the sample with binding buffer or by buffer exchange using PD-10 MiniTrap™ G-25 or HiTrap™ Desalting columns (see Section 8).
- Clarify the sample before applying it to the beads, if needed.
- Inhibiting protease activity in the sample prevents degradation of the target protein. A Protease Inhibitor Mix from GE Healthcare is available (see Section 8).

4 Protocol for capturing and eluting biotinylated proteins

This protocol is suitable for capturing and eluting biotinylated proteins. The first part of the protocol (1-3) captures biotinylated proteins from the sample and can be used for depletion.

1 Preparing the Mag Sepharose beads

A Mix the medium slurry thoroughly by vortexing. Dispense 100 µl of the homogenous medium slurry into an Eppendorf tube.

B Place the Eppendorf tube in the magnetic rack (see Section 2).

C Remove the storage solution.

2 Equilibrating

A Add 500 µl binding buffer and resuspend the medium.

B Remove the liquid.

3 Applying the sample

A Add 300 µl of sample. If the sample volume is less than 300 µl, dilute to 300 µl with binding buffer.

B Resuspend the medium and incubate for 30 minutes with slow end-over-end mixing or by using a benchtop shaker.

C Remove the sample.

4 Washing (perform this step three times totally)

A Add 500 µl washing buffer and resuspend the medium.

B Remove the liquid.

5 Eluting biotinylated proteins

A Add 100 µl elution buffer.

B Resuspend the medium and incubate at 95°C to 100°C for five minutes.

C Remove and collect the eluted fraction. The collected fraction contains the main part of the protein. If needed, repeat the elution.

Note: *The streptavidin-biotin bond can be broken efficiently only by harsh denaturing conditions. Hence, dissociation of biotin from streptavidin will denature both—the biotinylated protein and streptavidin, causing a leakage of the streptavidin monomers.*

5 Protocol for immunoprecipitation

This protocol is suitable as a starting point for enrichment of target proteins using immobilized antibodies.

1 Preparing the Mag Sepharose beads

A Mix the medium slurry thoroughly by vortexing. Dispense 50 µl of the homogenous medium slurry into an Eppendorf tube.

B Place the Eppendorf tube in the magnetic rack (see Section 2).

C Remove the storage solution.

2 Equilibrating

A Add 500 µl binding buffer and resuspend the medium.

B Remove the liquid.

3 Binding of biotinylated antibody

A Add 300 µl of biotinylated antibody solution (~0.2-0.4 mg/ml). If the sample volume is less than 300 µl, dilute to 300 µl with the binding buffer.

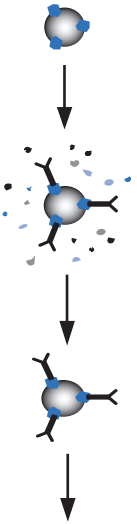
B Resuspend the medium and incubate for 30 minutes with slow end-over-end mixing or by using a benchtop shaker.

C Remove the liquid.

4 Washing (perform this step two times totally)

A Add 500 µl washing buffer and resuspend the medium.

B Remove the liquid.

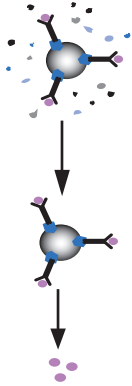


5 Binding of the target protein

A Add 300 µl of sample. If the sample volume is less than 300 µl, dilute to 300 µl with binding buffer.

B Resuspend the medium and incubate for 60 minutes with slow end-over-end mixing or by using a benchtop shaker.

C Remove the liquid.



6 Washing (perform this step three times totally)

A Add 500 µl washing buffer and resuspend the medium.

B Remove the liquid.

7 Eluting

A Add 50 µl of elution buffer.

B Resuspend the medium and incubate for two minutes.

C Remove and collect the elution fraction. The collected elution fraction contains the main part of the protein. If needed, repeat the elution.

6 Optimizing the parameters

The protocols recommended in this instruction (see Section 4 and Section 5) are suitable as starting points for most purifications involving biotinylated proteins. However, the optimal parameters for protein enrichment depend on the specific biomolecules used. Hence, optimization may be required to obtain the best results.

Examples of parameters which may require optimization are:

- Amount of beads
- Amount of biotinylated proteins/antibodies
- Amount of protein (antigen) to be enriched
- Incubation time
- Number of washes
- Buffer compositions, pH etc.

Non-specific binding can be minimized by decreasing the amount of Mag Sepharose beads, increasing the amount of biotinylated protein or by optimizing the wash conditions. If proteins are bound non-specifically to the Eppendorf tube, the bead solution can be transferred to a fresh tube during the last wash step.

7 Characteristics

Table 3. Streptavidin Mag Sepharose

Matrix	Highly cross-linked spherical agarose (Sepharose) containing magnetite
Medium	Streptavidin-coupled NHS activated Mag Sepharose
Ligand	Streptavidin
Binding capacity	> 300 µg biotinylated BSA/ml of medium slurry
Particle size	37-100 µm
Working temperature	Room temperature and at +4°C
Storage solution	20% ethanol, 10% medium slurry
Storage temperature	+4°C to +8°C

8 Ordering information

Products	Quantity	Code No.
Streptavidin Mag Sepharose	2 × 1 ml 10% medium slurry	28-9857-38
Streptavidin Mag Sepharose	5 × 1 ml 10% medium slurry	28-9857-99

Related products	Quantity	Code No.
MagRack 6	1	28-9489-64
MagRack Maxi	1	28-9864-41
His Mag Sepharose Ni	2 × 1 ml 5% medium slurry	28-9673-88
His Mag Sepharose Ni	5 × 1 ml 5% medium slurry	28-9673-90
His Mag Sepharose Ni	10 × 1 ml 5% medium slurry	28-9799-17
NHS Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-09
NHS Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-80
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 A Mag Sepharose Xtra	2 × 1 ml 10% medium slurry	28-9670-56
Protein A Mag Sepharose Xtra	5 × 1 ml 10% medium slurry	28-9670-62
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
Protein G Mag Sepharose Xtra	2 × 1 ml 10% medium slurry	28-9670-66

Related products	Quantity	Code No.
Protein G Mag Sepharose Xtra	5 × 1 ml 10% medium slurry	28-9670-70
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
HiTrap™ Desalting	5 × 5 ml	17-1408-01
PD MiniTrap™ G-25	50 columns	28-9180-07
Protease Inhibitor Mix	1 ml	80-6501-23
Streptavidin Buffer Kit	1	28-9135-68

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Protocol for immunoprecipitation

This protocol is suitable as a starting point for enrichment of target proteins using immobilized antibodies.

1 Preparing the Mag Sepharose beads

A Mix the medium slurry thoroughly by vortexing. Dispense 50 µl of the homogenous medium slurry into an Eppendorf tube.

B Place the Eppendorf tube in the magnetic rack (see Section 2).

C Remove the storage solution.

2 Equilibrating

A Add 500 µl binding buffer and resuspend the medium.

B Remove the liquid.

3 Binding of biotinylated antibody

A Add 300 µl of biotinylated antibody solution (~0.2-0.4 mg/ml). If the sample volume is less than 300 µl, dilute to 300 µl with the binding buffer.

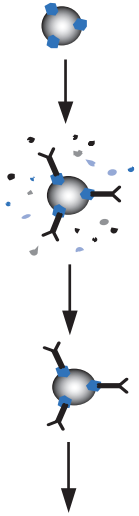
B Resuspend the medium and incubate for 30 minutes with slow end-over-end mixing or by using a benchtop shaker.

C Remove the liquid.

4 Washing (perform this step two times totally)

A Add 500 µl washing buffer and resuspend the medium.

B Remove the liquid.



5 Binding of the target protein

A Add 300 µl of sample. If the sample volume is less than 300 µl, dilute to 300 µl with binding buffer.

B Resuspend the medium and incubate for 60 minutes with slow end-over-end mixing or by using a benchtop shaker.

C Remove the liquid.

6 Washing (perform this step three times totally)

A Add 500 µl washing buffer and resuspend the medium.

B Remove the liquid.

7 Eluting

A Add 50 µl of elution buffer.

B Resuspend the medium and incubate for two minutes.

C Remove and collect the elution fraction. The collected elution fraction contains the main part of the protein. If needed, repeat the elution.