Streptavidin Mag **Sepharose**

PROTEIN SAMPLE PREPARATION

Streptavidin Mag Sepharose™ (Fig 1) is a magnetic bead for simple and efficient enrichment of target proteins by immunoprecipitation and purification of biotinylated biomolecules. Streptavidin Mag Sepharose utilizes the strong interaction between biotin and streptavidin ligand, which is immobilized on magnetic beads. Magnetic beads simplify sample handling in small-scale purifications.

Streptavidin Mag Sepharose delivers:

- High-capacity small-scale purification of proteins from cell lysates and biological fluids
- High purity and yield
- Easy parallel screening of proteins with high repeatability
- Simple enrichment of proteins from small or large sample volumes (low-microliter to high-milliliter scale)

The beads are available in two pack sizes: 2×1 ml 10% medium slurry and 5×1 ml 10% medium slurry. A milliliter of 10% medium slurry is the same as 100 µl sedimented medium and it is sufficient for 20 purification runs according to the recommended immunoprecipitation protocol.

Characteristics of the streptavidin-biotin bond

The binding of streptavidin to biotin is one of the strongest known noncovalent biological interactions. Hence, denaturing 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 molecules such as proteins. Impurities are removed by washing, and the enriched target protein is eluted using less harsh elution conditions.

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

Fig 1. Streptavidin Mag Sepharose is designed for enrichment of target proteins by immunoprecipitation or purification of biotinylated biomolecules.

ensures reliable collection of the beads in the purification procedure. Streptavidin Mag Sepharose is provided with two protocols; one for capturing and eluting biotinylated proteins and one for immunoprecipitation. The characteristics of the media are summarized in Table 1.

Table 1. Characteristics of 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 to 100 µm
Working temperature	Room temperature and at 4°C
Storage solution	20% ethanol, 10% medium slurry
Storage temperature	4°C to 8°C

*The binding capacity is protein dependent.

Magnetic racks such as MagRack 6 or MagRack Maxi (Fig 2) are excellent complements to Mag Sepharose products from Cytiva. MagRack 6 allows you to prepare up to six samples captured in 1.5 ml microcentrifuge tubes and MagRack Maxi can be used for sample volumes up to 50 ml. The magnetic beads are attracted to the magnet within a few seconds when the tubes are placed in the rack. This allows you to easily remove the supernatant while the magnetic beads are retained in the tube. In addition, you can easily screen a large number of samples with high-throughput on a robotic device.

Fig 2. The high density of the beads allows rapid capture by MagRack 6 (left) and MagRack Maxi (right) magnetic devices.

The use of Streptavidin Buffer Kit allows you to eliminate tedious buffer preparations and this leads to fast and reproducible purification.

Efficient enrichment by immunoprecipitation

Streptavidin Mag Sepharose was used to enrich a sample of human transferrin spiked *E. coli* lysate. The concentration of transferrin comprised 0.15% of the total *E. coli* protein content, which corresponds to medium level protein expression in *E. coli.* Capture of the protein of interest was achieved using a biotinylated polyclonal rabbit anti-human transferrin immobilized on the medium (Table 2). SDS-PAGE analysis of the first and second elution fractions revealed a transferrin recovery of 75% and a 450-fold enrichment relative to the start material. The first elution step contained 75% of the purified protein (Fig 3).

Table 2. Experimental conditions for Streptavidin Mag Sepharose

Medium slurry volume	50 µl
Sample	7.5 µg/ml human transferrin in 5 mg/ml *E. coli* protein
Sample volume	0.3 ml
Antibody	Polyclonal rabbit anti-human transferrin (biotinylated)
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	100 mM glycine-HCl, 2 M urea, pH 2.9

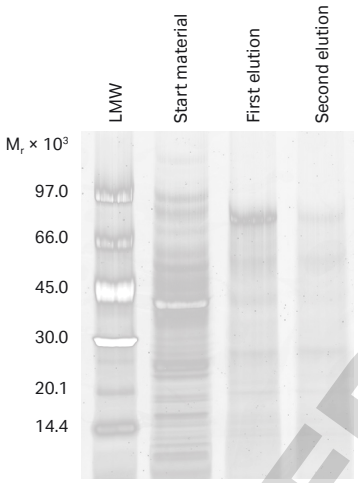

Fig 3. Efficient enrichment of transferrin (M$_r$ 80 000) spiked in *E. coli* lysate. The SDS gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant™ TL software. Quantitation of the eluted transferrin was performed using standard curves with known amounts of transferrin (data not shown). LMW = Low molecular weight protein markers.

Repeatable capture of biotinylated BSA

In order to test the repeatability of Streptavidin Mag Sepharose, we performed six replicate runs (Table 3) with a sample load corresponding to 80% of the total binding capacity for the media. Protein recovery was estimated by SDS-PAGE analysis of the eluted fractions in triplicate. The SDS-PAGE gel was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software. We found that protein recovery with Streptavidin Mag Sepharose was consistently high (> 75%) and highly repeatable with a relative standard deviation (RSD) of 4% (Fig 4).

Table 3. Experimental conditions for Streptavidin Mag Sepharose

Medium slurry volume	100 µl
Sample	Pure biotinylated bovine serum albumin (BSA)
Sample volume	300 µl
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% SDS at 95°C

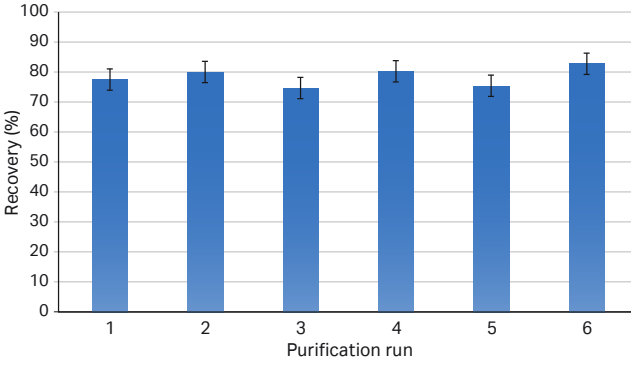

Fig 4. The recovery for six replicate purification runs (analyzed in triplicate) was consistently > 75% and the variation was 4% (RSD). The error bars represent the 95% confidence interval of the SDS-PAGE analysis.

Comparison of the binding capacities of various streptavidin magnetic beads

We conducted a comparative benchmark analysis at Cytiva laboratories to investigate the binding capacity for biotinylated polyclonal rabbit IgG using different streptavidin magnetic beads products. We compared the performance of Streptavidin Mag Sepharose with corresponding magnetic bead products from Qiagen™, Thermo Scientific™, and Invitrogen™.

If a purification protocol was supplied for the product, capture of the biotinylated protein was performed according to each manufacturer's instructions. In the absence of a supplied protocol, we used the protocol for Streptavidin Mag Sepharose to perform the capture step (Table 4). A slurry (100 µl) of each media was used to determine the binding capacity and all the media were subjected to an overloading test, (i.e., the load of IgG was above the capacity for each media).

The results show that Streptavidin Mag Sepharose has considerably higher binding capacity for biotinylated IgG than the corresponding products from Qiagen, Thermo Scientific, and Invitrogen. Figure 5 shows that approximately 170 µg IgG was bound to 100 µl Streptavidin Mag Sepharose. Hence, the binding capacity of 1 ml medium slurry was 1.7 mg/ml medium slurry.

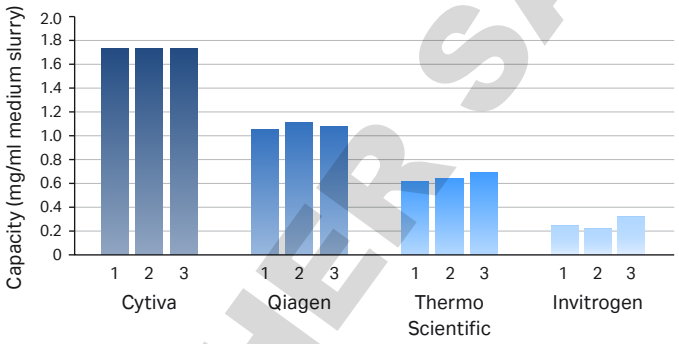

Fig 5. Binding capacities for polyclonal rabbit IgG using different streptavidin magnetic beads. The binding capacity was calculated by subtracting the amount of protein in the flowthrough from the amount of protein loaded. We determined protein concentration with BCA Protein Assay. Each bar represents the amount of IgG determined for a 1 ml medium slurry.

Table 4. Experimental conditions for determing the binding capacities of various streptavidin magnetic beads

Supplier	Cytiva	Qiagen	Thermo Scientific	Invitrogen
Separation media	Streptavidin Mag Sepharose	BioMag™ Streptavidin	Pierce™ Streptavidin Magnetic Beads	Dynabeads™ MyOne™ Streptavidin T1
Medium slurry volume	100 µl	100 µl	100 µl	100 µl
Load	Overloaded	Overloaded	Overloaded	Overloaded
Binding buffer	Tris-buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.5)	TBS, pH 7.5	TBS + 0.1% Tween 20, pH 7.5	Phosphate buffered saline (PBS: 140 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4)

Comparison of protein enrichment using various streptavidin magnetic beads

We compared the extent of protein enrichment using Streptavidin Mag Sepharose to that of corresponding magnetic beads products from Qiagen, Thermo Scientific, and Invitrogen (Fig 6). The study was conducted at Cytiva laboratories by immunoprecipitation of 7.5 µg/ml transferrin spiked in 5 mg/ml of *E. coli* lysate using a biotinylated polyclonal rabbit antibody immobilized on the media. A slurry (50 µl) of each media was used to capture the antibody and immunoprecipitation was performed in duplicate according to the manufacturer's protocol. If a purification protocol was supplied for the product, enrichment of transferrin was performed according to each manufacturer's instructions. In the absence of a supplied protocol, we used the protocol for Streptavidin Mag Sepharose to perform the enrichment (Table 5).

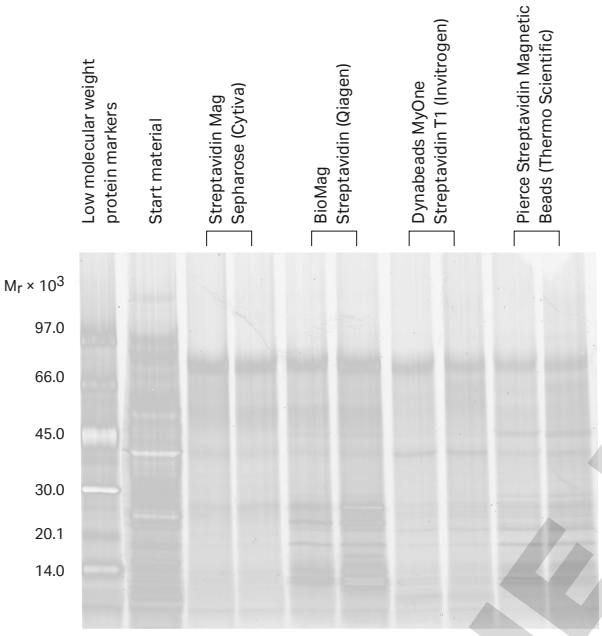

Fig 6. SDS-PAGE analysis of eluates from the immunoprecipitation of transferrin (M_r 80 000). The SDS gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software.

A comparison of the degree of protein enrichment (Fig 7) shows that human transferrin was enriched 430-fold using Streptavidin Mag Sepharose, 300-fold using Dynabeads MyOne Streptavidin T1 (Invitrogen), 150-fold using BioMag Streptavidin (Qiagen) and 120-fold using Pierce Streptavidin Magnetic Beads (Thermo Scientific).

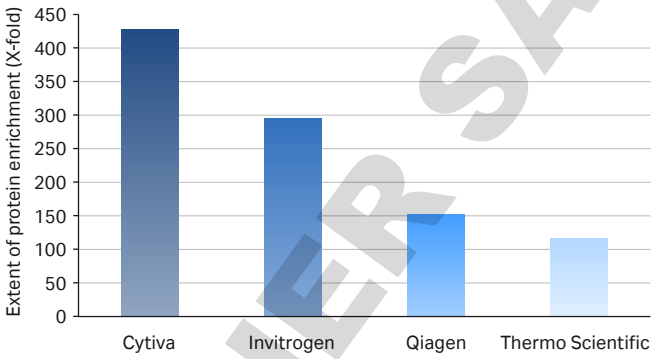

Fig 7. Enrichment of human transferrin using streptavidin magnetic beads from different suppliers. We used SDS gel electrophoresis to determine the degree of enrichment of transferrin. The SDS gel (reducing conditions) was stained with Deep Purple Total Protein Stain and analyzed with ImageQuant TL software.

Table 5. Experimental conditions for the analysis of purified samples with various magnetic beads

Supplier	Cytiva	Qiagen	Invitrogen	Thermo Scientific
Separation media	Streptavidin Mag Sepharose	BioMag Streptavidin	Dynabeads MyOne Streptavidin T1	Pierce Streptavidin Magnetic Beads
Medium slurry volume	50 µl	50 µl	50 µl	50 µl
Antibody load	0.3 ml of 0.2 mg/ml	0.3 ml of 0.2 mg/ml	0.3 ml of 0.2 mg/ml	0.3 ml of 0.033 mg/ml
Sample	0.3 ml of 7.5 µg/ml human transferrin in 5 mg/ml *E. coli* protein			
Binding buffer	Tris-buffered saline (TBS: 50 mM Tris, 150 mM NaCl) pH 7.5	TBS	Phosphate-buffered saline (PBS: 140 mM NaCl, 2.7 mM KCl, 10 mM phosphate), pH 7.4	TBS + 0.1% Tween 20
Wash buffer	TBS + 2 M urea, pH 7.5	TBS + 2 M urea, pH 7.5	PBS	TBS + 0.1% Tween 20
Elution buffer	0.1 M glycine-HCl + 2M urea, pH 2.9			

Immunoprecipitation of low concentration transferrin from large sample volumes

The ability to use different volumes of sample and medium slurry is one of the key advantages of the magnetic beads separation method. We used Streptavidin Mag Sepharose to purify human transferrin spiked in 5 mg/ml of *E. coli* lysate. Purification was scaled up 10-fold from 50 µl to 500 µl of Streptavidin Mag Sepharose and 3 ml and 30 ml of sample, respectively. The experiment was performed in duplicate using MagRack Maxi (Table 6). The transferrin concentration was 0.75 µg/ml, which corresponds to ~ 0.015% of total *E. coli* protein content. Transferrin was captured by immunoprecipitation using a biotinylated polyclonal rabbit anti-human transferrin immobilized on the medium and we estimated the yield of transferrin by SDS gel electrophoresis of the eluted fractions.

The results (Fig 8) shows the inherent flexibility of Streptavidin Mag Sepharose. We obtained a corresponding increase in the purity and recovery of transferrin when we scaled up immunoprecipitation 10-fold. The yields of transferrin were 1.2 µg and 13 µg using 50 µl and 500 µl of Streptavidin Mag Sepharose slurry, respectively. The average purity was 78% (5200-fold enrichment) and 75% (5000-fold enrichment).

Table 6. Experimental conditions for Streptavidin Mag Sepharose

Medium slurry volume	50 µl or 500 µl
Sample	0.75 µg/ml human transferrin in 5 mg/ml *E. coli* protein
Sample volume	3 or 30 ml
Antibody	Polyclonal rabbit anti-human transferrin (biotinylated)
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	100 mM glycine-HCl, pH 2.9

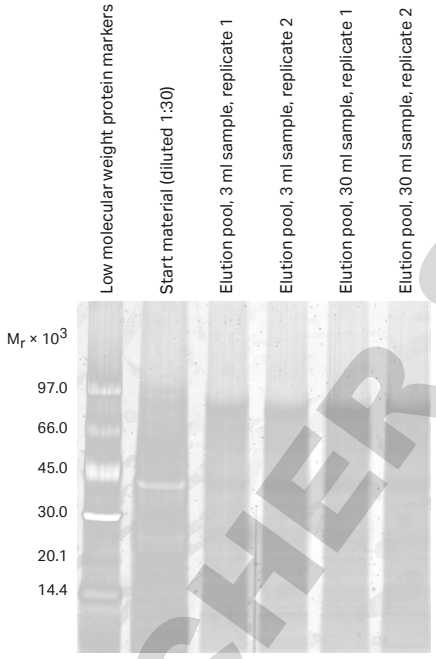

Fig 8. SDS-PAGE (reducing conditions) stained with Deep Purple Total Protein Stain. The purity and recovery obtained were equally high when the scale of purification was increased 10-fold. Quantitation of the eluted transferrin was performed using standard curves with known amounts of transferrin (data not shown).

Ordering information

Products	Quantity	Code number
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 number
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
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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