

A benchmark study of streptavidin magnetic beads

T. Granér, M. Björner, and A. Bergh

GE Healthcare Bio-Sciences AB, Uppsala, Sweden

The binding of streptavidin to biotin is one of the strongest known noncovalent biological interactions and hence a powerful tool for immobilization of ligands for affinity chromatography. Starting with the same streptavidin medium, the desired affinity can be achieved by immobilization of any biospecific molecule derivatized with biotin. This medium can then be used for purification of the desired target. The use of magnetic beads for affinity-based purification simplifies small-scale purifications and provides high flexibility with scales ranging from microliter to milliliter. In this study, we compare the magnetic bead Streptavidin Mag Sepharose™ with corresponding products from other companies. For the tested proteins, Streptavidin Mag Sepharose offers leading performance in terms of binding capacity and achieved enrichment.



Binding capacity for biotinylated IgG

A comparative benchmark analysis was performed at GE Healthcare Life Sciences' laboratories to investigate the binding capacity of different streptavidin magnetic beads products for biotinylated polyclonal rabbit IgG (DakoCytomation). The antibody was biotinylated using EZ-Link™ Sulfo-NHS-LC Biotinylation kit (Thermo Scientific™). We compared the

performance of Streptavidin Mag Sepharose with corresponding magnetic beads from Qiagen™, Thermo Scientific, and Invitrogen™ (Table 1). When a purification protocol was supplied for the product, capture of the biotinylated antibody was performed according to each manufacturer's instructions. If no protocol was supplied, capture was performed as for Streptavidin Mag Sepharose. To determine the binding capacity, 100 µl of medium slurry of each medium was overloaded with IgG and the binding capacity was calculated by subtracting the amount of protein collected in the flowthrough fractions from the amount of protein loaded as determined using the BCA Protein Assay (Thermo Scientific). The determinations were performed in triplicate.

Binding capacity of Streptavidin Mag Sepharose was approx. 1.7 mg IgG/ml of medium slurry, which is considerably higher than the binding capacity of the other products.

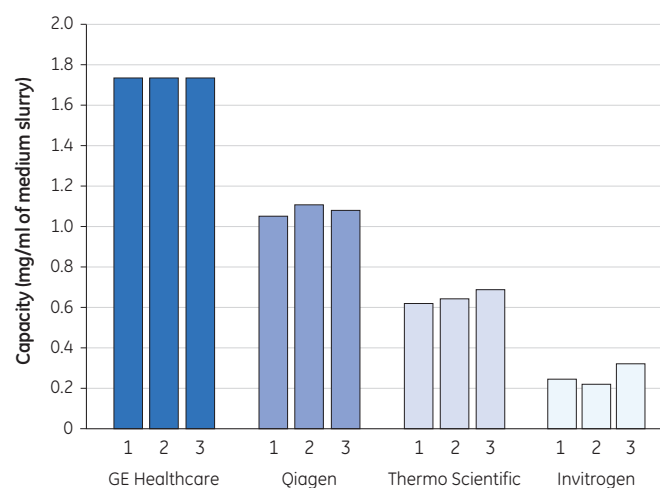


Fig 1. Binding capacity for biotinylated polyclonal rabbit IgG for four different streptavidin magnetic beads. Each bar represents the amount of IgG that can be captured using 1 ml of medium slurry.

Table 1. Experimental conditions for binding capacity determination

Supplier	GE Healthcare	Qiagen	Thermo Scientific	Invitrogen
Separation medium	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

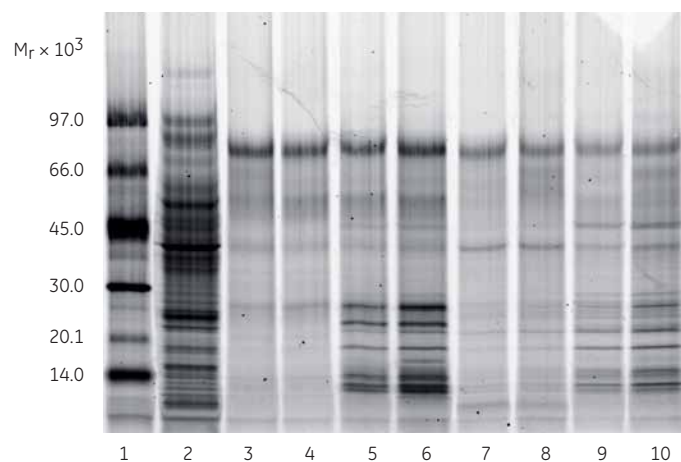
Table 2. Experimental conditions purity assessment

Supplier	GE Healthcare*	Qiagen*	Thermo Scientific*	Invitrogen*
Separation medium	Streptavidin Mag Sepharose	BioMag Streptavidin	Pierce Streptavidin Magnetic Beads	Dynabeads MyOne Streptavidin T1
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 <i>E.coli</i> protein			
Binding buffer	TBS, pH 7.5	TBS, pH 7.5	TBS, 0.1% Tween 20, pH 7.5	PBS
Wash buffer	TBS, 2 M urea, pH 7.5	TBS, 2 M urea, pH 7.5	TBS, 0.1% Tween 20, pH 7.5	PBS
Elution buffer	0.1 M glycine-HCl, 2 M urea, pH 2.9			

* Manufacturer's recommended protocols were used throughout this study

Enrichment of captured proteins

In another comparative study, the enrichment of proteins captured on streptavidin magnetic beads with immobilized biotinylated IgG antibodies was determined. Streptavidin Mag Sepharose was compared with corresponding magnetic beads from Qiagen, Thermo Scientific, and Invitrogen as in the binding capacity test. A biotinylated polyclonal rabbit antibody was immobilized on 50 µl of medium slurry of each medium, after which they were exposed to 300 µl of 7.5 µg/ml of human transferrin in a background of 5 mg/ml *E. coli* proteins. This immunoprecipitation procedure was performed in duplicate following the manufacturer's protocol when supplied (Table 2). The enrichment of the eluted fractions was estimated by SDS-PAGE (Fig 2) and the SDS gels were analyzed using ImageQuant™ TL software (Fig 3). Streptavidin Mag Sepharose gave a 430-fold enrichment; higher than for Invitrogen (300-fold), Qiagen (150-fold), and Thermo Scientific (120-fold).



Lane

- 1 Low molecular weight markers
- 2 7.5 µg/ml transferrin in 5 mg/ml *E. coli* protein
- 3, 4 Eluted pool: Streptavidin Mag Sepharose
- 5, 6 Eluted pool: BioMag Streptavidin
- 7, 8 Eluted pool: Dynabeads MyOne Streptavidin T1
- 9, 10 Eluted pool: Pierce Streptavidin magnetic beads

Fig 2. Purity benchmark study. The SDS gel was run under reducing conditions, stained with Deep Purple™ Total Protein Stain, and analyzed using ImageQuant TL software.

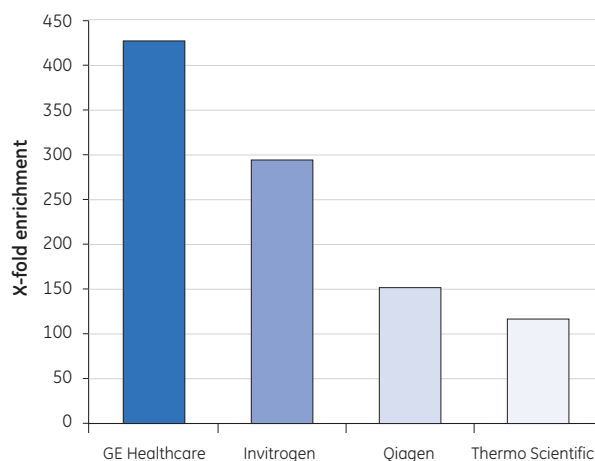


Fig 3. Enrichment of human transferrin using streptavidin magnetic beads from different suppliers. Enrichment of transferrin relative to the start material was determined by SDS-PAGE analysis. Quantitation of the eluted transferrin was performed using a standard curve with known amounts of transferrin (data not shown).

Conclusions

A benchmark study performed at our laboratory showed that Streptavidin Mag Sepharose has considerably higher binding capacity for biotinylated IgG as compared to the tested streptavidin magnetic beads products from Qiagen, Thermo Scientific, and Invitrogen.

Moreover, the enrichment achieved using Streptavidin Mag Sepharose beads was better than the other products tested. In the test system, an enrichment factor of 430 was obtained with Streptavidin Mag Sepharose, which was higher than for the equivalent products from other suppliers.

Ordering information

Product	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

For more information on Mag Sepharose beads for protein enrichment and immunoprecipitation, visit www.gelifesciences.com/sampleprep