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ProductInformation

MagSelect™ SA

Product Number **M 0444**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Synonym: Streptavidin Magnetic Agarose Beads

Product Description
Streptavidin, a 66 kDa homotetrameric protein isolated from *Streptomyces avidinii*, is similar to avidin with respect to its high affinity for biotin ($K_a \sim 10^{15}\ M^{-1}$).[1-4] Streptavidin is slightly anionic (pI ~5.6) and non-glycosylated - both properties contributing to the relatively low non-specific binding compared to egg white avidin (a glycoprotein with pI ~10.5).

The binding of biotinylated biomolecules to streptavidin is virtually irreversible. Very harsh conditions including low pH and denaturants are needed to completely remove the biotinylated molecules. This attribute makes MagSelect™ SA beads very useful in a variety of affinity capture applications. Examples include capture of biotinylated macromolecules and complexes such as proteins, antibodies, lectins, nucleic acids, receptors, and ligands.[5-11] MagSelect SA beads can also be used for direct purification of desthiobiotinylated proteins and peptides. This allows the molecule of interest to be eluted from the beads under mild conditions, using competitive displacement with biotin.[12]

MagSelect SA is composed of streptavidin attached to paramagnetic iron impregnated 6% agarose beads, with an average diameter of 50 µm. The resulting linkage has an effective spacer length of 14 atoms. The magnetic properties allow for very rapid separation of the beads from a suspension, significantly accelerating manipulations, such as repetitive washings, or processing of multiple samples performed in multiwell plates. That leads to faster experimentation, better reproducibility, and more accurate quantitation of the proteins of interest.

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Binding capacity:
- Minimum 175 nmoles of biotin per ml of chromatographic medium
- minimum 2 mg of biotinylated BSA per ml of chromatographic medium

Specificity:
MagSelect SA has been tested for specificity of binding biotinylated proteins. Non-specific binding of 5% or less is observed when the reagent is used in a RIPA Buffer system (Product Code R 0278).

Reagents
MagSelect SA is supplied as a 50% suspension in 0.01 M sodium phosphate, pH 7.2, containing 50% glycerol, 0.15 M NaCl, and 15 ppm Kathon® CG/ICP II (as a preservative).

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
For ease of handling, the glycerol concentration may be reduced by dilution with a neutral buffer such as Phosphate Buffered Saline, pH 7.4, (PBS, Product Code P 3813) prior to use.

Equilibration for Batch Format - For the procedure described in this bulletin, 50 µl (100 µl of suspension) of MagSelect SA is utilized. Transfer the required amount of resin slurry to an appropriately sized microcentrifuge tube. When pipetting, use a wide orifice tip or cut ~1 mm off the end of a regular pipette tip to allow for unrestricted flow of the bead suspension. To equilibrate the medium, add 5 volumes of PBS, mix, and centrifuge for 30 seconds at 2,000 *x g* (e.g., 5,000 rpm in a Eppendorf® 5415 C microcentrifuge). Carefully remove the supernatant using a micropipette. Repeat the equilibration step two more times.

Storage/Stability
MagSelect SA ships on wet ice and storage at 2–8 °C is recommended. The product, as supplied in 50% glycerol with preservative, may be stored at –20 °C. However, freezing the magnetic beads in the absence of 50% glycerol will irreversibly damage the bead structure. For long term storage of the medium following equilibration of the beads and removal of glycerol, the medium should be stored at 2–8 °C with a preservative (e.g. 15 ppm of Kathon CG/ICP II, Product Code 48178U) and **not frozen**.

Procedures

A. Procedure for Immunoaffinity Batch Purification Using a Biotinylated Antibody as a Ligand

1. Transfer the required amount of equilibrated medium (see Preparation Instructions) to an appropriately sized microcentrifuge tube. For this procedure, 50 µl of MagSelect SA is utilized.

2. Add the biotinylated antibody dissolved in an appropriate neutral buffer such as PBS. To prevent non-specific binding of non-biotinylated molecules, detergents or blocking agents may also be included in the solution. Fifty µl of MagSelect SA will bind ~50 µg of a biotinylated antibody. For optimal binding, incubate with mixing for a minimum of 15 minutes at room temperature.

3. Wash away any unbound antibody with PBS. Specifically, add 500 µl of PBS, then mix and place on an appropriate magnetic device for 20 seconds. Remove the supernatant. Repeat this step five more times.

4. Apply the sample (i.e. mixture containing antigen) to the microcentrifuge tube. For optimal binding, incubate with mixing for a minimum of 15 minutes at room temperature.

5. Wash away any unbound biomolecules with PBS. Specifically, add 500 µl of PBS then mix and place on an appropriate magnetic device for 20 seconds. Remove the supernatant. Repeat this step five more times.

6. Elute the sample (i.e. antigen) with 0.1 M acetic acid or 0.1 M glycine HCl (pH 2.5) or other elution buffer to dissociate the antibody-antigen binding interaction. Immediately neutralize eluted samples, which are now ready for further analysis.

B. Purification of Desthiobiotinylated Biomolecules in a Centrifuge Tube Batch Format

1. Transfer the required amount of equilibrated medium (see Preparation Instructions) to an appropriately sized microcentrifuge tube. For this procedure, 50 µl of MagSelect SA is used.

2. Add up to 12 nmoles of desthiobiotinylated sample, dissolved in an appropriate neutral buffer such as PBS. To facilitate optimal binding, incubate with mixing for a period of at least 15 minutes at room temperature.

3. Wash away any unbound biomolecules with PBS. Specifically, add 500 µl of PBS then mix and place on an appropriate magnetic device for 20 seconds. Remove the supernatant. Repeat this step five more times.

4. To elute the desthiobiotinylated biomolecules, add 200 µl of 2 mM biotin in PBS. Allow the solution to incubate at room temperature with mixing for at least 15 minutes.

5. Place on an appropriate magnetic device, then remove and save the supernatant containing the desthiobiotinylated biomolecules of interest for further analysis.

Related Products	**Product Code**
Anti-FLAG® BioM2 Antibody	F 9291
Protein A – Biotin	P 2165
Protein G – Biotin	P 8045
Desthiobiotin PEO Iodoacetamide	D 2192
Magnetic separators for microcentrifuge tubes	M 1167
Magnetic separators for tissue culture flasks	M 1292
Magnetic separators for centrifuge tubes	M 1542

References

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