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Product Information

ExtrAvidin®-Agarose, high binding
for purification of biotinylated macromolecules and complexes

Catalog Number **E2513**
Storage Temperature 2–8 °C

Product Description

ExtrAvidin® is a unique deglycosylated, modified form of an affinity purified egg white avidin. The ExtrAvidin is conjugated to cyanogen bromide-activated agarose beads at ~4 mg protein per ml of settled resin.

ExtrAvidin®-Agarose provides high affinity with high specificity binding to biotin (also known as vitamin B7) including biotinylated proteins and biotin-tagged fusion proteins. The product may be used in various immunological techniques, including immuno-precipitation and immunoaffinity purification.

ExtrAvidin is a tetrameric protein containing four biotin binding sites. The avidin-biotin high affinity interaction ($K_d = 10^{-15}$ M) is considered one of the strongest non-covalent interactions known in nature.[1-3] The use of the avidin-biotin complex for affinity purification was described in the first half of 1970 and since then it was successfully utilized in numerous studies and biotechnological applications.[1-3]

This was achieved due to the ability to chemically couple the small biotin molecule with different binders, without disturbing its function or structure, thus allowing the unique interaction with a variety of avidin carriers including: protein or DNA molecules, avidin protein bound to a solid surface matrix, reporter molecules, probes or carriers. Applications of the avidin-biotin interaction include: purification, enrichment, detection, amplification, and other research medical and industrial processes.[1-7] In addition, the ExtrAvidin high specificity binding to biotin, together with the low background staining, give it significant advantage compared to the non-modified avidin or streptavidin produced by *Streptomyces avidinii*.

ExtrAvidin-Agarose is provided as suspension at a 1:1 ratio, in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Binding capacity: ≥600 nmoles of biotin per 1 ml of settled resin.
Note: Binding capacity and elution yields may vary depending on the characteristics of the biotin-tagged fusion protein. For optimal results it is recommended to test different elution buffers.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

In order remove the sodium azide in the storage buffer, the agarose beads must be washed thoroughly with 5-10 column volumes of buffer.

Storage/Stability

For continuous use and extended storage, store at 2–8 °C. **Do not freeze**.

Procedure

Notes: These procedures are provided as general guidelines. Appropriate and optimal conditions should be determined empirically by the end user for any specific application. It is also recommended to determine the working concentration by a titration test.

Procedure for Column Purification of Biotinylated Proteins or Biotinylated Antibodies

1. Add the ExtrAvidin-Agarose beads into an appropriate column and wash 3-5 times with 5-10 beads volume of PBS.
2. Apply the sample containing the biotinylated protein/biotinylated antibody and incubate for 30 minutes at room temperature.
3. Wash with PBS until the absorbance at 280 nm is minimal.

4. Elution of biotinylated proteins using one of the following steps:
   - Boiling for 5 minutes with 2% SDS in 0.4 M urea or with 1× SDS Sample Buffer, followed by SDS-PAGE analysis.
   - Incubation for 30 minutes at room temperature with 2 M acetic acid or 2 M glycine HCl, pH 2.2. If desired, immediately neutralize the eluted samples.

References

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