

Product Information

# ExtrAvidin® Magnetic beads, high binding

For purification of biotinylated macromolecules and complexes

#### E2642

# **Product Description**

ExtrAvidin® is a tetrameric protein containing four biotin binding sites. The avidin-biotin high affinity interaction ( $K_d = 10^{-15} \, \text{M}$ ) is considered one of the strongest non-covalent interactions known in nature. The use of the avidin-biotin complex for affinity purification was described in 1970. It has since been used in numerous studies and biotechnological applications. This was achieved because of the ability to couple chemically biotin with different binders, without disturbing its function or structure. This allows 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.

Avidin-biotin interaction-based applications include purification, enrichment, detection, amplification and other research medical and industrial processes. <sup>1-7</sup> In addition, the ExtrAvidin® high specificity binding to biotin, together with the low background staining, grants it a significant advantage compared to non-modified avidin or streptavidin produced by *Streptomyces avidinii*.

ExtrAvidin®-Magnetic beads are a unique, de-glycosylated, avidin-modified form of an affinity-purified egg white avidin. The ExtrAvidin® is conjugated to cyanogen bromide-activated magnetic beads at a ratio of ~4 mg/mL protein-to-beads.

ExtrAvidin®-Magnetic beads provide 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 immunoprecipitation and immunoaffinity purification.

# Reagent

ExtrAvidin®-Magnetic beads are provided as a suspension containing 50% beads, in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

## 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.

# Storage/Stability

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

## **Product Profile**

1

**Binding capacity:** at least 650 nmoles of biotin per 1 mL of magnetic beads-settled resin.

Recommended Procedure for Purification of Biotinylated Proteins or Biotinylated Antibodies

Pre-washing ExtrAvidin®-Magnetic beads: To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform.

- 1. Add 200  $\mu$ L of ExtrAvidin®-Magnetic beads into a 1.5 mL microcentrifuge tube.
- 2. Place the tube into a magnetic stand to collect the beads against the side of the tube.
- 3. Remove and discard the supernatant. Wash with 200 µL PBS. Repeat the washing 3-5 times.
- 4. Apply the sample containing the biotinylated protein/antibody at  $\sim$ 750 µg/test. Incubate for 30 min at RT using a rotating platform.
- 5. Wash with PBS until the absorbance at 280 nm in minimal. Save flow-through for calculating the binding capacity.



E2642 Rev 02/22

- 6. Elution of biotinylated proteins:
  - Boiling for 5 min with 1× SDS sample buffer, followed by SDS-PAGE analysis, or:
  - Incubation with 2 M acetic acid or 2 M Glycine HCL pH 2.2. If required, immediately neutralize eluted samples.

**Note:** In order to obtain the best results in different techniques and preparations, we recommend determining the optimal working concentration by a titration test.

Binding capacity and elution capacity may vary, depending on the characteristics of the Biotin-tagged fusion proteins. For optimal results, it is recommended to try different elution buffers.

#### References

- 1. Wilchek, M., and Bayer, E.A., *Trends Biochem. Sci.*, **14(10)**, 408-412 (1989).
- Bayer, E.A., and Wilchek, M., J. Chromatogr., 510, 3-11 (1990).
- Wilchek, M., Protein Sci., 13(11), 3066-3070 (2004).
- 4. O'Connor, E. et al., J Immunol Methods., **229(1-2)**, 155-160 (1999).
- 5. Gao, H. et al., Proc. Natl. Acad. Sci. USA, **105(51)**, 20146-20151 (2008).
- 6. Iikura, M. *et al.*, *J. Leukoc. Biol.*, **70(1)**, 113-120 (2001).
- Kellenberger, L.D., and Petrik, J., Gynecol. Oncol., 149(2), 361-370 (2018).

### Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

#### Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

## Standard Warranty

The applicable warranty for the products listed in this publication may be found at <a href="SigmaAldrich.com/terms">SigmaAldrich.com/terms</a>.

#### **Contact Information**

For the location of the office nearest you, go to SigmaAldrich.com/offices.

The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.



2