CaptAvidin™ Biotin-Binding Protein

C-21385 CaptAvidin™ biotin-binding protein
C-21386 CaptAvidin™ agarose *sedimented bead suspension*
C-21387 CaptAvidin™ acrylamide

Quick Facts

Storage upon receipt:

C-21385, C-21387:

- –20°C
- Desiccate

C-21386:

- 4°C
- Do not freeze

Introduction

The high affinity and specificity of avidin–biotin interactions have been exploited for diverse applications in immunology, histochemistry, *in situ* hybridization, affinity chromatography and in many other areas.[1-3] The avidin–biotin interaction is a very strong non-covalent bond with a K_a of 10^{15} M^{-1}.[4] Although this high affinity binding is advantageous for most applications, it becomes a major drawback for affinity chromatography. The conditions needed to dissociate the avidin–biotin complex (8 M guanidine hydrochloride, pH 1.5) are usually too harsh for most proteins and prevent the use of avidin for purifying biotinylated molecules.

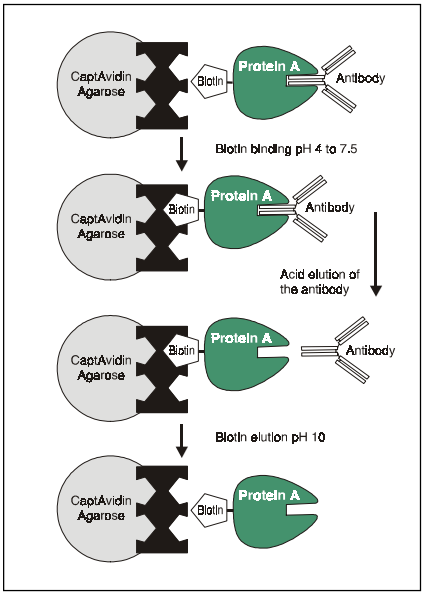

Figure 1. *Diagram of the use of CaptAvidin agarose in affinity chromatography. In this example, biotinylated protein A and an IgG molecule are used as the target.*

Molecular Probes now offers a derivative of avidin, CaptAvidin™ biotin-binding protein [5] (C-21385), in which the tyrosine in the biotin-binding site is nitrated. This chemical modification permits dissociation of the avidin–biotin complex under conditions more compatible with protein purification. At pH 4.0, CaptAvidin biotin-binding protein binds biotin tightly with a K_a of ~10^9 M^{-1}.[6] This association can then be reversed at pH 10, allowing complete dissociation of the avidin–biotin complex (Figure 1). This ability to reverse the biotin binding to the CaptAvidin biotin-binding protein allows researchers to use the reagent multiple times.

In addition, we offer CaptAvidin biotin-binding protein conjugated to agarose (C-21386) for use in affinity chromatography. This form of agarose-immobilized biotin-binding protein has been used to purify immunoglobulin from whole rabbit serum and to isolate anti-transferrin antibodies directly from rabbit IgG fractions.[7]

CaptAvidin biotin-binding protein conjugated to acrylamide (C-21387) is another reagent that has potential use for biotinylated-probe isolation. CaptAvidin biotin-binding protein acrylamide can be co-polymerized with acrylamide on polymeric surfaces to create a uniform monolayer of the immobilized protein.[8,9] The CaptAvidin biotin-binding protein will then bind biotinylated-ligands, including hybridization probes, enzymes, antibodies and drugs. The biotinylated-probes can subsequently be dissociated from the CaptAvidin biotin-binding protein using basic pH conditions.

Materials

CaptAvidin biotin-binding protein and CaptAvidin biotin-binding protein acrylamide conjugate are supplied in unit sizes of 1 mg and should be stored desiccated at -20°C. Solutions of 2 mg/mL can be prepared by dissolving the powder in PBS or other suitable buffers. Once in solution the products should be divided into smaller aliquots and stored at -20°C. AVOID REPEATED FREEZING AND THAWING.

CaptAvidin biotin-binding protein conjugate of 4% beaded, cross-linked agarose (Sepharose® CL-4B) is supplied in a unit size of 5 mL of sedimented gel in phosphate-buffered saline,

pH 7.2 with 5 mM sodium azide and should be stored at 4°C. DO NOT FREEZE.

All non-nitrated biotin-binding sites of the CaptAvidin biotin-binding protein have been pre-blocked with free biotin so that these irreversible sites will not interact with biotinylated molecules. The binding capacity of the CaptAvidin biotin-binding protein is then measured using fluorescein biotin (B-1370). Typically, 1 µg of CaptAvidin biotin-binding protein binds 200–250 ng of biotin. For the CaptAvidin biotin-binding protein agarose conjugate, approximately 0.35 mg of fluorescein biotin (~420 nmole) binds per mL of sedimented gel.

Applications

Purification of Immunoglobulin with Biotinylated Protein A

This is a protocol for purification of immunoglobulin using biotinylated protein A and CaptAvidin agarose in a slurry.[7]

1.1 Suspend 2 mL of CaptAvidin sedimented agarose in 2 mL of biotin-binding buffer (50 mM citrate phosphate buffer, pH 4.0). Mix well.

1.2 Add 1.8 mg of biotinylated protein A (dissolved in biotin-binding buffer, pH 4.0) to the slurry. Gently rock the tube at room temperature for 15 minutes. Centrifuge at 3000 × g for 15 minutes and discard supernatant.

1.3 Wash the sedimented agarose with 4 mL of IgG-binding buffer (50 mM Tris-HCl, pH 8.0). Centrifuge and discard the supernatant.

1.4 Dilute 0.5 mL of rabbit serum with 1.5 mL of IgG-binding buffer. Add this to the CaptAvidin agarose slurry. Gently rock the tube at room temperature for 15 minutes.

1.5 Centrifuge and discard the supernatant.

1.6 Wash the sedimented agarose with 4 mL of 10 mM Tris-HCl buffer, pH 8.0. (Please note that the molarity of this buffer is fivefold less than that of the IgG-binding buffer used in steps 1.3 and 1.4.) Centrifuge and discard the supernatant.

1.7 Remove the IgG from the protein A–CaptAvidin biotin-binding protein complex by adding 1–2 mL of IgG-extraction buffer (50 mM citrate phosphate buffer, pH 4.0). Gently rock the tube at room temperature for 15 minutes.

1.8 Centrifuge and save the supernatant, which contains the isolated IgG.

1.9 Wash the CaptAvidin agarose slurry 2 times in 2 mL of the same pH 4.0 buffer. (Save these supernatants until the recovery of IgG in the first supernatant is determined.)

1.10 Elute the biotinylated protein A from the CaptAvidin agarose by adding 2 mL of elution buffer (50 mM sodium carbonate-HCl buffer, pH 10) to the agarose slurry. Gently rock the tube at room temperature for 15 minutes. Centrifuge and save the supernatant containing the biotinylated protein A.

1.11 Wash the CaptAvidin agarose slurry 2 times in 2 mL of the same pH 10 buffer. (Save these supernatants until the recovery of the protein A in the first supernatant is determined.)

1.12 Wash the CaptAvidin biotin-binding protein agarose slurry 2 times in 4 mL of phosphate-buffered saline (PBS), pH 7.2. Discard the supernatants. Resuspend the agarose in 2 mL of PBS, pH 7.2 containing 2 mM sodium azide. Store at 4°C.

The above protocol can be modified for column purification of IgG. To increase the bed volume of the column, mix 2 mL of CaptAvidin agarose with 2 mL of a 4% suspension of Sepharose® CL-4B agarose in either PBS or biotin-binding buffer (50 mM citrate phosphate buffer, pH 4.0).

References

1. Methods Enzymol 184, (complete volume) (1990); **2.** Anal Biochem 171, 1 (1988); **3.** Methods Biochem Anal 26, 1 (1980); **4.** Adv Protein Chem 29, 85 (1975); **5.** Licensed from Baxter Healthcare Corporation under U.S. patent No. 5,973,124; **6.** Biochem J 316, 193 (1996); **7.** Anal Biochem 243, 257 (1996); **8.** Biotechniques 27, 592 (1999); **9.** Nucleic Acids Res 27, 649 (1999).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
C-21387	CaptAvidin™ acrylamide	1 mg
C-21386	CaptAvidin™ agarose *sedimented bead suspension*	5 ml
C-21385	CaptAvidin™ biotin-binding protein	1 mg

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Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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