USER GUIDE

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Streptavidin Agarose

For affinity chromatography and immunoprecipitation of biotinylated proteins

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Important Information

Shipping and Storage

The streptavidin agarose is shipped and should be stored at 4°C. Streptavidin agarose is guaranteed for six months from the date of receipt if stored properly.

Note: Avoid freezing as this may result in loss of activity.

Contents

Streptavidin agarose is supplied as 10 mL of a 50% slurry containing 5 mL of packed gel bed in 0.01 M sodium phosphate, pH 7.2, 0.15 M NaCl, and 0.05% sodium azide.

For instructions on how to obtain safety information and a Safety Data Sheet (SDS) for sodium azide, refer to **Technical Support**, page 13.

Conjugation

Streptavidin is covalently linked to crosslinked agarose beads via a 15-atom hydrophilic spacer arm to produce streptavidin agarose. The specially designed spacer arm reduces non-specific binding and ensures optimal binding of biotinylated molecules. Streptavidin is bound to a final concentration of 2–3 mg of streptavidin per mL of packed gel.

Applications

The streptavidin agarose is suitable for use in the following applications:

- Affinity chromatography to isolate and purify biotinylated molecules (Haeuptle et al., 1983)
- Immunoprecipitation (e.g., immunoprecipitation using antibodies which have a low affinity for protein A including some mouse and rat monoclonal antibodies (Updyke and Nicolson, 1984))

BioEase™ Biotinylation System

A number of vectors are available from Life Technologies to facilitate production of a biotinylated protein of interest. The vectors contain a 72 amino acid BioEase™ tag, which when fused to a heterologous protein of interest, allows *in vivo* biotinylation of the recombinant fusion protein (Schwarz et al., 1988). For more information about the vectors available, see our website (**www.lifetechnologies.com**) or call Technical Support (see page 13).

Methods

Overview

Introduction

The streptavidin agarose utilizes the high affinity streptavidin-biotin interaction to allow immune-precipitation and affinity chromatography of biotinylated proteins (Bayer, 1968; Wilchek and Bayer, 1984).

Streptavidin-Biotin Interaction

Streptavidin is an avidin analog isolated from culture filtrates of *Streptomyces avidinii* (Chaiet and Wolf, 1964) that binds biotin with extremely high affinity, but exhibits little of avidin's non-specific binding at physiological pH. This is presumed to be due to streptavidin's:

- Low isoelectric point
- Lack of glycosylation

Streptavidin can be covalently bound to a solid phase support (i.e., agarose beads) with no effect on its biotin-binding activity or low non-specific binding characteristics (Haeuptle et al., 1983; Updyke and Nicolson, 1984).

Important

We have found that the streptavidin-biotin complex is essentially covalent and **is not dissociable** even under the stringent elution conditions recommended in the literature (Heney and Orr, 1981). While this tight association may be useful for some applications (e.g., immunoprecipitation), it can restrict the use of the streptavidin-agarose for other applications, particularly affinity isolation and purification.

In those cases where reversible binding is desired, we recommend using a biotin analog such as iminobiotin. Iminobiotin exhibits a pH-dependent interaction with streptavidin, and will bind to streptavidin at high pH (>9.5) and dissociate at low pH (<4.0) (Hofmann et al., 1982; Orr, 1981).

Biotinylating Proteins

A number of methods are available to biotinylate your protein of interest:

1. You may express your protein of interest using one of the vectors in the BioEase™ Biotinylation System available from Life Technologies. These vectors allow fusion of your protein of interest to a BioEase™ tag, which facilitates *in vivo* biotinylation of your recombinant fusion protein. For more information about the options available, visit our website (**www.lifetechnologies.com**) or call Technical Support (see page 13).
2. You may conjugate biotin (or iminobiotin) directly to your protein of interest (e.g., antibodies, ligands, or other biological molecules) (Guesdon et al., 1979). For a procedure to biotinylate an antibody, see the **Appendix**, page 11.

General Guidelines for Use

Buffer Systems

Streptavidin-agarose is supplied in a standard phosphate-buffered saline. Depending on your needs, you may alter the buffer system without affecting the binding capabilities of the resin. For example, we have found that the matrix is stable in the presence of detergents (i.e., NP40, Triton X-100, or SDS).

Note: Tris and HEPES buffered systems may be substituted for phosphate-buffered saline without affecting the binding ability of the streptavidin-agarose.

Washing the Resin

Immediately prior to use, thoroughly wash the streptavidin-agarose with the buffer which will be used in the experiment. Wash with approximately 100X the volume of resin to be used.

Dispensing the Resin

When pipetting streptavidin-agarose, we recommend using large bore, disposable plastic pipette tips. To ensure reproducible dispensing, cut 3 mm from the end of each pipette tip before use.

Affinity Chromatography

Introduction

Streptavidin-agarose may be used in affinity chromatography applications to isolate, purify, and characterize biotinylated proteins. Depending on the nature of your biotinylated protein, you will need to determine the optimal buffer system and conditions to use to purify your biotinylated protein. General guidelines for use are provided below. If you are purifying BioEase™ tag-containing fusion proteins from *E. coli*, additional guidelines are provided on page 9.

General Guidelines

Consider the following when using streptavidin-agarose for affinity chromatography applications:

- Binding can be performed in batch mode or in a column. Remember to wash and equilibrate the resin with the buffer that will be used in the experiment immediately prior to use.
- Biotinylated proteins may be isolated under native or denaturing conditions. **Note:** We have recovered functional biotinylated protein when using denaturants such as guanidine HCl or urea (see page 9).
- Purification can be performed using whole cell lysates or culture medium containing secreted, biotinylated protein expressed from bacterial, mammalian, or insect cells. If you are using large volumes of culture medium, we recommend first using size exclusion filtration to concentrate the medium and remove low molecular weight components.

Affinity Chromatography, Continued

Eluting Biotinylated Proteins

Because of the extremely strong interaction between streptavidin and biotin, biotinylated proteins cannot be dissociated from the streptavidin-agarose even under stringent conditions (Heney and Orr, 1981). We have successfully eluted biotinylated proteins from streptavidin-agarose by cleavage using an enterokinase such as EnterokinaseMax™ (EKMax™) available from Life Technologies (Cat. no. E180-01).

To elute a biotinylated protein from streptavidin-agarose using EKMax™ enterokinase:

1. Equilibrate the bound resin (i.e., streptavidin-agarose-biotinylated protein complexes) in EKMax™ Buffer.
2. Incubate the bound resin overight at room temperature in EKMax™ Buffer and 250–500 units of EKMax™ enzyme per mL of resin.
3. The following day, elute the cleaved protein by collecting the supernatant. **Note:** To remove EKMax™ enzyme, you may use EK-Away™ Resin available from Life Technologies (Cat. no. R180-01).

Purifying BioEase™ Tag-Containing Fusion Proteins from *E. coli*

Additional guidelines are provided in this section to facilitate purification of BioEase™ tag-containing fusion proteins expressed in *E. coli* using Life Technologies' Champion™ pET104 BioEase™ Gateway® Biotinylation System (Cat. no. K104-01).

- **Starting Point:** We generally use a 50 mL bacterial culture to prepare 1 mL of whole cell lysate, which is applied to a 1 mL bed volume of streptavidin-agarose. Volumes may be varied according to your needs.
- Recombinant, biotinylated proteins expressed in *E. coli* are often insoluble. We have found that purifying the proteins using mildly denaturing conditions (i.e., 1–3 M guanidine HCl or 2–4 M urea) can facilitate increased capture and recovery.

Immunoprecipitation

Introduction

Use the provided guidelines to immunoprecipitate biotinylated antibodies with streptavidin-agarose. Other proteins may be immunoprecipitated using streptavidin-agarose, but parameters may need to be varied to obtain the optimal conditions for detection.

Immuno-precipitation Procedure

Use the following procedure to immunoprecipitate biotinylated antibody with streptavidin-agarose. **Note:** For immuno-precipitations involving secondary biotinylated antibodies, we recommend increasing the amount of streptavidin-agarose used 3-fold if quantitative recovery is required.

1. Thoroughly wash the streptavidin-agarose using the appropriate buffer. Wash with approximately 100X the volume of resin to be used.
2. Pretreat antigen-containing sample for endogenous biotin (e.g., biotinyl-enzymes) by incubating with 2 µL of streptavidin-agarose (1 µL of packed gel volume) per 100 µL of sample for 30 minutes. After treatment, place sample in a microcentrifuge and centrifuge for 10 seconds.
3. Transfer supernatant to a sterile microcentrifuge tube.
4. Incubate antigen-containing sample with approximately 10 µg of biotinylated antibody per 100 µL of sample using conditions suitable for your antibody-antigen system. Ensure thorough mixing of the sample during incubation. **Note:** Common incubation conditions are 1–3 hours at room temperature or on ice.
5. Add 20 µL of streptavidin-agarose (10 µL of packed gel volume) to the sample. Incubate for 30 minutes at room temperature with continuous mixing. Keep the streptavidin-agarose suspended during incubation by frequent vortexing (every 5 minutes) or by placing samples on an end-over-end rotator.
6. Place samples in a microcentrifuge and centrifuge for 10 seconds.
7. Wash gel pellet 3–4 times with the appropriate buffer.
8. Process the streptavidin-agarose containing bound antigen-biotinylated antibody complex as desired.

Appendix

Biotinylating Antibodies

Introduction

Use the procedure below to conjugate biotin to a purified antibody of interest or to another protein of interest (Guesdon et al., 1979). In most cases, this procedure will yield an antibody that is sufficiently biotinylated for efficient use with streptavidin-agarose.

Materials Needed

Have the following materials on hand before beginning:

- Purified antibody or other protein of interest
- Phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
- Biotin-N-hydroxysuccinimide ester or caproylamido-biotin-N-hydroxysuccinimide ester (CAB-NHS)
- Dimethylformamide (store in a dessicator until use)
- 0.5 M sodium carbonate buffer, pH 9.0
- 1 M NH_4Cl
- Dialysis reagents and buffer of choice

Biotinylating Antibodies, Continued

Biotinylation Procedure

Use this procedure to conjugate biotin to a purified antibody or other protein of interest.

1. Adjust concentration of purified antibody to 1–10 mg/mL in PBS.
2. Dissolve biotin-N-hydroxysuccinimide ester or CAB-NHS to a concentration of 50 mg/mL in dimethyl-formamide.
3. Adjust antibody solution to pH 9 with 0.5 M sodium carbonate buffer, pH 9.0.
4. Incubate biotin ester and purified antibody at a ratio of 1:2.5 to 1:10 (w:w) biotin ester:protein for 1–4 hours at room temperature.
5. Add 1 M NH_4Cl to a final concentration of 0.1 M in the reaction mixture to stop the reaction.
6. Dialyze extensively against the buffer of choice to remove free biotin.
7. Compare the biological activity of the biotinylated antibody with the non-biotinylated antibody to ensure that activity is retained.

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
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- Obtain information about customer training
- Download software updates and patches

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Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit
lifetechnologies.com/support or email **techsupport@lifetech.com**

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