SNAP-Capture Pull Down Resin

1-800-632-7799
info@neb.com
www.neb.com

S9144S

2 ml **Lot: 0041106**

Store at: 4°C **Exp: 6/13**

Introduction

The SNAP-Capture Pull Down Resin is used to selectively capture and immobilize a SNAP-tag® fusion protein from solution using agarose beads. These beads have a high loading capacity for SNAP-tag fusion proteins and show very low non-specific absorption of proteins from a complex lysate, making them especially suitable for pull down applications. The SNAP-Capture Pull Down Resin is prepared by the coupling of SNAP-tag substrate benzylguanine with a highly cross-linked agarose (4%) with excellent physical characteristics. Two ml of SNAP-Capture Pull Down Resin is sufficient to perform 24 pull down assays using 80 µl of resin suspension per assay.

The SNAP-tag is a novel tool for protein research, allowing the specific, covalent attachment of virtually any molecule or modified surface to a protein of interest. The SNAP-tag is a polypeptide based on mammalian O^6-alkylguanine-DNA-alkyltransferase (AGT). SNAP-tag substrates are derivates of benzyl purines and benzyl pyrimidines. In the immobilization reaction, the SNAP-tag is covalently attached to the substituted benzyl group of the SNAP-Capture Pull Down Resin.

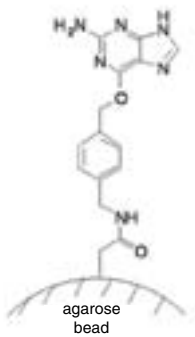

Figure 1. *Substrate structure on SNAP-Capture Pull Down Resin*

There are two steps to the use of this system: sub-cloning and expression of the protein of interest as a SNAP-tag fusion, and capture and immobilization of the fusion protein using SNAP-Capture Pull Down Resin. Expression of SNAP-tag fusion proteins is described on www.neb.com. The immobilization of the fusion proteins to SNAP-Capture Pull Down Resin is described here. An example of a pull down protocol can also be found on www.neb.com.

Materials Required but not Supplied

Protein sample containing the protein to immobilize expressed as a SNAP-tag fusion

Buffer for immobilization and washing

Microcentrifuge

Storage of Pull Down Resin

Do not freeze the SNAP-Capture Pull Down Resin. Store the unused resin at 4°C. With proper storage, resin should be stable for at least one year.

Quality Control

Binding Capacity: SNAP-Capture Pull Down Resin (80 µl) was washed, incubated with 200 µl of 1 mg/ml SNAP-tag CBD (Chitin Binding Domain) for 1 hour at 37°C, then rewashed as described in these instructions. Binding capacity was determined to be 3 mg/ml bed resin.

Instructions for Use

This protocol describes the use of SNAP-Capture Pull Down Resin in small scale batch format. It can be scaled-up to fit the requirements for specific applications.

Protocol for Equilibration of Resin

The SNAP-Capture Pull Down Resins are stored in 50% isopropyl alcohol. The storage buffer must be exchanged with immobilization buffer before use.

1. Carefully and thoroughly resuspend the 25% SNAP-Capture Pull Down Resin suspension. Immediately withdraw an 80 µl sample to a 1.5 ml microcentrifuge tube. The 80 µl sample contains 20 µl bed-volume resin.
2. Add 1 ml of immobilization buffer to the resin. Vortex gently to mix. Centrifuge for 1 minute at 11000 x g. Carefully remove and discard the supernatant, resuspend and repeat 2 times.

Protocol for Immobilization of SNAP-tag Fusion Protein

Prepare a protein solution containing up to 1 mg/ml SNAP-tag fusion protein to be immobilized in an appropriate buffer containing at least 1 mM DTT. We recommend the use of at least 100 µl of this solution for each immobilization reaction.

1. Add this protein solution to the SNAP-Capture Pull Down Resin in a 1.5 ml microcentrifuge tube (20 µl bed-volume resin) prepared as above.
2. Incubate with mixing for 1 hour at room temperature. Alternatively, incubate overnight at 4°C with mixing.

Protocol for Washing Step

The washing step removes non-specifically bound protein after the immobilization reaction.

1. Wash the resin at least three times with 1 ml of immobilization buffer. To wash, add immobilization buffer to the resin and agitate for one minute, centrifuge (1 minute at 11,000 x g at 4°C), carefully remove and discard the supernatant, and repeat.
2. The SNAP-Capture Pull Down Resin immobilized fusion protein is now ready for further use (e.g. pull down assay). See www.neb.com for an example protocol.

Usage Notes

Storage and Handling of SNAP-tag Fusion Proteins

Correct storage and handling of SNAP-tag fusion proteins is essential to maintain reactivity of the SNAP-tag prior to immobilization.

SNAP-tag fusion proteins can be purified before immobilization, but the immobilization reaction also works in non-purified protein solutions including cell lysates.

Add 1 mM DTT to buffers used for the storage of SNAP-tag fusion proteins. Protein samples should be stored at –20°C, or at –80°C for long-term storage. Handling at temperatures above 0°C should be minimized by thawing the SNAP-tag protein samples shortly before use, and keeping them on ice until just before the immobilization.

If a particular fusion protein requires buffers without reducing agents, pay particular attention to minimize all handling steps of the protein above 4°C before the labeling reaction.

The SNAP-tag itself is tolerant of a wide range of buffers. The requirements of your fusion partner should dictate the selection of the buffer. The following storage buffer composition is recommended, especially when freezing protein material: pH between 7.0 and 8.0, monovalent salts (e.g. sodium chloride) between 50 mM and 250 mM, and at least 1 mM DTT. Non-ionic detergents can be added if required, but ionic detergents should be avoided because they reduce the activity of the SNAP-tag. Many proteins benefit from the addition of glycerol for frozen storage, typically 20% v/v.

Storage and Handling of Immobilized SNAP-tag Fusion Proteins

Immobilized SNAP-tag fusion proteins should be stored at 4°C. Sodium azide may be added to 2 mM final concentration to prevent bacterial growth. Depending on the stability of the fusion partner, under these conditions the immobilized protein should be stable at 2–6°C for several months. The SNAP-Capture Magnetic Beads should not be frozen.

The SNAP-tag fusion protein is linked to the SNAP-Capture Pull Down Resin by a covalent bond. Therefore the immobilized protein is essentially irreversibly bound to the resin. It is important however to preserve the functional stability of the protein fused to the SNAP-tag as much as possible. We recommend handling the immobilized fusion protein and storing between use at 4°C, to prepare it just before use, and to handle it as gently as possible.

Troubleshooting

If sufficient immobilization of a protein sample is not achieved using the recommended conditions, try the following protocol modifications: Double the incubation time to two hours total at 25°C or to 24 hours at 4°C. If you still have poor immobilization results, we recommend checking the activity of the SNAP-tag (see below).

If your fusion protein is particularly sensitive to degradation or to loss of activity, you can try reducing the immobilization time or decreasing the immobilization temperature. If you immobilize at 4°C we recommend overnight incubation. Addition of a standard EDTA-free protease inhibitor cocktail, although not generally necessary for the SNAP-tag itself, may also help.

(see other side)

CERTIFICATE OF ANALYSIS

The activity of the SNAP-tag may have been partially or completely lost. This may be due to extended storage of non-reacted SNAP-tag fusion proteins at 4°C or above. The sensitivity of the SNAP-tag to inactivation is decreased if a reducing agent such as 1 mM DTT is added.

If the activity of the SNAP-tag is affected, we recommend analyzing a small fraction of it on a PAGE gel using SNAP-Vista to confirm that the SNAP-tag is active.

If you encounter problems with the activity we recommend thawing another sample of your protein or reexpressing and repurifying the SNAP-tag fusion protein following the advice given in the SNAP-tag plasmid instructions.

Washing Step

It is important to maintain the functional integrity of the SNAP-tag fusion protein during the washing step. Although the SNAP-tag is covalently linked to SNAP-Capture Pull Down Resin, and will remain bound under harsh conditions, we recommend washing the beads before use only under mild buffer conditions to minimize the possible loss of fusion protein function. Fusion protein stability is highly protein dependent.

Some partially purified fusion proteins may exist as multimers and higher molecular weight aggregates. It may not be possible to wash aggregates away under mild conditions. This would not interfere with most applications but these proteins could leach off the resin under extreme denaturing conditions (e.g. SDS).

Protein Interaction Assay

Depending on the strength and properties of the particular interacting proteins in a protein interaction study, the following post-interaction washing parameters can be varied to improve the specificity of removing weakly interacting proteins; salt concentration (100–500 mM), non-ionic detergents (0.1–1%), glycerol (10–30%), DTT (0.1–1 mM) or EDTA (1–10 mM).

Addition of a standard protease inhibitor cocktail, although not generally necessary for the SNAP-tag itself, may be useful to prevent degradation of the interacting proteins when working with complex mixtures such as lysate.