SNAP-Capture Magnetic Beads

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S9145S 004120214022

S9145S

2 ml | Lot: 0041202

Store at: 4°C | Exp: 2/14

Introduction

The SNAP-Capture Magnetic Beads are used to selectively immobilize and magnetically separate a SNAP-tag® fusion protein from solution using magnetic agarose beads. These beads show a low non-specific absorption of proteins from a complex lysate, making them suitable for pull down applications. The SNAP-Capture Magnetic Beads are prepared by the coupling of SNAP-tag substrate benzylguanine with highly stable 75–150 µm superparamagnetic particles. Two ml of SNAP-Capture Magnetic Beads is sufficient to perform 25 immobilization assays using 80 µl of bead 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 protein based on mammalian O^6-alkylguanine-DNA alkyltransferase (AGT). SNAP-tag substrates are derivatives 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 Magnetic Beads.

There are two steps to using this system: subcloning and expression of the protein of interest as a SNAP-tag fusion, and capture and immobilization of the fusion protein using SNAP-Capture Magnetic Beads. Protocols for expression of SNAP-tag fusion proteins are supplied with SNAP-tag plasmids. A protocol for immobilization of fusion proteins to SNAP-Capture Magnetic Beads is included here. An example of a pull down protocol can be found at www.neb.com.

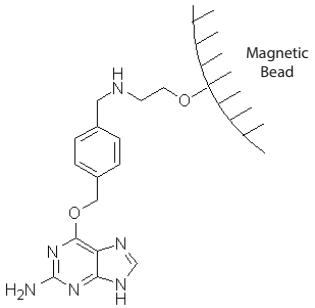

Figure 1. Substrate structure on SNAP-capture Magnetic Beads.

Materials Required but not Supplied

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

Magnetic particle separator

Buffer for immobilization and washing

Storage of Magnetic Beads

Do not freeze the magnetic beads. Store the unused beads at 4°C. With proper storage, beads should be stable for at least two years.

Quality Control

Binding Capacity: SNAP-Capture Magnetic Beads (80 µl) were washed, incubated with 200 µl of 1 mg/ml SNAP-tag CBD (Chitin Binding Domain) for 1 hour at room temperature, then washed as described in these instructions. Binding capacity was determined to be 3 mg/ml bead suspension.

Instructions for Use

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

Protocol for Equilibration of Beads

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

1. Carefully and thoroughly resuspend the SNAP-Capture Magnetic Beads suspension. Immediately withdraw an 80 µl sample to a 1.5 ml microcentrifuge tube.
2. To equilibrate the beads add 1 ml of immobilization buffer to the beads and vortex gently.

   The immobilization buffer should be the optimal buffer for the stability of your protein. The SNAP-tag is active between pH 5–10. Salt concentration should be adjusted for your particular fusion protein. SNAP-tag fusions have been shown to be stable from 150 mM to 1 M monovalent salt (e.g. NaCl). If the protein is especially hydrophobic, the addition of 0.05% to 0.1% Tween 20 may help. We also recommend the addition of 1 mM DTT to all buffers used for handling, immobilization and storage of the SNAP-tag fusion protein. Non-ionic detergents can be added to 0.5% v/v if required, but SDS and other ionic detergents should be avoided entirely because they inhibit the activity of the SNAP-tag. Metal chelating reagents (e.g. EDTA and EGTA) also inhibit SNAP-tag activity and should be avoided. You can bring the buffer up in water or 1X PBS.

   Place the microcentrifuge tube on a magnetic particle separator. Separate the beads from solution, carefully remove and discard the supernatant, and repeat once.

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 immobilization 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 Magnetic Beads in a 1.5 ml microcentrifuge tube.
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. To wash, add 1 ml of immobilization buffer, agitate for one minute, separate beads from solution using magnetic particle separator for ten seconds, carefully remove and discard the supernatant. Repeat twice.
2. The SNAP-Capture Magnetic Beads immobilized fusion protein is now ready for further use (e.g. pull down assay). An example protocol can be found at www.neb.com.

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, minimize 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 Magnetic Beads by a covalent bond. Therefore the immobilized protein is essentially irreversibly bound to the beads. 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.

(see other side)

CERTIFICATE OF ANALYSIS

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.

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 by addition of a reducing reagent such as 1 mM DTT.

If the activity of the SNAP-tag is affected, we recommend analyzing a small fraction of it on a PAGE gel using SNAP-Vista Green (NEB #S9147) 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. While the SNAP-tag is covalently linked to SNAP-Capture Magnetic Beads, 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 beads 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.

Companion Products Sold Separately:
6-Tube Magnetic Separation Rack

#S1506S	6 tubes