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Use SNAP-Capture Magnetic Beads (S9145)

Materials Required but not Supplied

SNAP-Capture Magnetic Beads

- Protein sample containing the protein to immobilize expressed as a SNAP-tag fusion
- Magnetic particle separator
- Buffer for immobilization and washing

Overview

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

Equilibration of Resin

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 50% 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. Place the microcentrifuge tube on a magnetic particle separator (Magnetic Separation Rack, NEB# S1506). Separate the beads from solution, carefully remove and discard the supernatant, and repeat once.

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 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.

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).

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.