Labeling of Proteins in Solution (S9221)

Overview

Dissolve the vial of CLIP-Biotin (50 nmol) in 17 µl of DMSO to yield a stock solution of 3 mM CLIP-tag substrate in DMSO. Mix for 10 minutes until all the CLIP-tag substrate is dissolved.

Protocol

1. Prepare a protein solution containing up to 20 µM CLIP-tag fusion protein to be labeled in an appropriate buffer containing at least 1 mM DTT.
2. Add 3 mM CLIP-tag substrate solution to a total volume of 1% of the volume of the protein solution. Carefully pipette the material up and down to mix, and vortex briefly.
3. Incubate for 1 hour at 25°C in the dark. Alternatively incubate overnight at 4°C in the dark.

   Biotin labeled CLIP-tag proteins can be used for Western Blot analysis. Alternatively they can be used to study protein interaction in interaction assays or used in pull-down assays. Biotinylated proteins can also be immobilized on streptavidin beads or on streptavidin plates.

   Removal of Unreacted Substrate (optional)
   After the labeling reaction you may wish to separate the unreacted substrate from the biotinylated CLIP-tag fusion protein before using it for your experiment. You can use size-exclusion columns or extensive dialysis. Please refer to the vendor's instructions for the separation tools you are using. Removal of unreacted substrate should not be necessary prior to detection of biotin labeled CLIP-tag fusion proteins on Western blots.

   Notes for Labeling in Solution

   Labeling
   We recommend the routine addition of 1 mM DTT to all buffers used for handling, labeling and storage of the CLIP-tag. The stability of the CLIP-tag is improved in the presence of reducing agents; however it can also be labeled in their absence.

   CLIP-tag fusion proteins can be purified before labeling, but the labeling reaction also works in non-purified protein solutions (including cell lysates).

   Confirmation of Labeling by Western Blot Analysis
   Labeled CLIP-tag fusion proteins can be easily analyzed on a SDS-PAGE gel/Western blot analysis because the covalently bound label will remain attached to the protein. The Biotin-label can be detected on an SDS-PAGE gel followed by Western Blot using a Horseradish Peroxidase or Alkaline Phosphatase labeled avidin/streptavidin (e.g. streptavidin-HRP) and the corresponding detection method as described by the supplier of the enyzme conjugate.

   Troubleshooting for Labeling in Solution

   Labeling Reaction
   If solubility problems occur with your SNAP-tag fusion protein, we recommend testing a range of pH (pH 5.0–pH 10.0) and ionic strengths. The salt concentration may also need to be optimized for your particular fusion protein (50–250 mM).

   If stickiness of the fusion protein is a problem we recommend adding Tween 20 at a final concentration of 0.05% to 0.1%.

The SNAP-tag activity is not affected by this concentration of Tween 20.

If exhaustive labeling 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; or halve the volume of protein solution labeled (50 µl of a solution containing up to 20 µM SNAP-tag fusion protein). Both approaches may be combined. If you still have poor labeling results, we recommend checking the activity of the SNAP-tag using SNAP-Vista.

If your fusion protein is particularly sensitive to degradation or to loss of activity, you can try reducing the labeling time or decreasing the labeling temperature. If you label at 4°C we recommend overnight incubation.