Labeling Proteins in Solution (S9135)

Overview

Dissolve the vial of SNAP-tag substrate (50 nmol) in 17 µl of DMSO to yield a stock solution of 3 mM SNAP-tag substrate. Pipette up and down periodically for 10 minutes until all the SNAP-tag substrate is dissolved.

Protocol

1. Prepare a protein solution containing up to 20 µM SNAP-tag fusion protein to be labeled in an appropriate buffer containing at least 1 mM DTT. We recommend labeling at least 100 µl of protein solution per experiment.
2. Add SNAP-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.
   Removal of Unreacted Substrate (optional)

   After the labeling reaction the unreacted substrate can be separated from the labeled SNAP-tag fusion protein by gel filtration or dialysis. Please refer to the vendor's instructions for the separation tools you are using.

   Notes
   We recommend the routine addition of 1 mM DTT to all buffers for used for handling, labeling and storage of the SNAP-tag. The stability of the SNAP-tag is improved in the presence of reducing agents; however it can also be labeled in their absence.
   SNAP-tag fusion proteins can be purified before labeling, but the labeling reaction also works in non-purified protein solutions (including cell lystates).

   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-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 over night incubation.