Labeling of Proteins *in vitro* (S9217)

Materials Required but not Supplied

CLIP-Cell™ 505

- Cells expressing CLIP-tag fusion proteins
- Tissue culture materials and media
- Transfection reagents
- Fluorescence microscope with suitable filter set
- DMSO

Overview

Protocol

1. Dissolve the vial of CLIP-Cell 505 substrate (50 nmol) in 50 µl of DMSO to yield a stock solution of 1 mM CLIP-tag substrate. Mix by vortexing for 10 minutes until all the CLIP-tag substrate is dissolved. Dilute this 1 mM stock solution 1:4 in fresh DMSO to yield a 250 µM stock for labeling proteins *in vitro*.

2. Set up the reactions, in order, as follows:

Component	Volume	Final Concentration
Phosphate Buffered Saline (PBS)	42 µl	1X
50 mM DTT	1 µl	1 mM
50 µM CLIP-tag Purified Protein	5 µl	5 µM
250 µM CLIP-tag Substrate	2 µl	10 µM
Total Volume	50 µl	

3. Incubate in the dark for 60 minutes at 37°C.

4. Run sample on an SDS-PAGE gel and detect using a fluorescent gel scanner or store samples at -20°C or -80°C in the dark.

Removal of Unreacted Substrate (optional)
After the labeling reaction the unreacted substrate can be separated from the labeled CLIP-tag fusion protein by gel filtration or dialysis. Please refer to the vendor's instructions for the separation tools you are using.

Notes for Labeling *in vitro*
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, if handling at temperatures above 4°C is minimized.

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

Troubleshooting for Labeling *in vitro*

Solubility

If solubility problems occur with your CLIP-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).

Loss of Protein Due to Aggregation or Sticking to Tube

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

Incomplete Labeling

If exhaustive labeling of a protein sample is not achieved using the recommended conditions, try the following protocol modifications: Increase 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. Both approaches may be combined. If you still have poor labeling results, we recommend checking the activity of the CLIP-tag using CLIP-Vista Green.

If the CLIP-tag fusion has been stored in the absence of DTT or other reducing agent, or has been stored at 4°C for a prolonged period, its activity may be compromised. Include 1 mM DTT in all solutions of the CLIP-tag fusion protein, and store the fusion protein at -20°C.

Using less than the recommended amount of substrate stock solution can significantly slow down the reaction rate.

Loss of Activity of Protein of Interest

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.