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Labeling on the Surface of Cells (S9350)

Overview

ACP-tag and MCP-tag fusion proteins are expressed by transient transfection. For expression of fusion proteins with the ACP-tag and MCP-tag, refer to instructions supplied with the pACP-tag and pMCP-tag plasmids. For cell culture and transfection methods, refer to established protocols.

Dissolve one vial of CoA substrate (50 nmol) in 50 µl of DMSO to give a labeling stock solution of 1 mM CoA substrate. Mix by vortexing for 10 minutes until all the substrate is dissolved. Store this stock solution in the dark at 4°C, or for extended storage at -20°C. Different stock concentrations can be made, depending on the requirements. The substrate is soluble up to at least 10 mM.

Protocol

1. Dilute the substrate stock solution 1:200 in medium to a final concentration of 5 µM. Mix dye with medium thoroughly by pipetting up and down 10 times (necessary for reducing backgrounds). For best performance, add the CoA substrate to complete medium, including serum (0.5% BSA can be used for experiments carried out in serum-free medium). Add $MgCl_2$ to a final concentration of 10 mM. Finally, add ACP Synthase or SFP Synthase to a final concentration of 1 µM, a dilution of 1:40. Do not prepare more medium with substrate, $MgCl_2$, and synthase than will be consumed within one hour.
2. Replace the medium on the cells expressing an ACP-tag or MCP-tag fusion protein with the labeling medium and incubate at 37°C, 5% CO_2 for 60 minutes.

Number of wells in plate	Recommended Volume for Cell Labeling
6	1 ml
12	500 µl
24	250 µl
48	100 µl
96	50 µl

3. Wash the cells three times with tissue culture medium with serum.
4. Image the cells using an appropriate filter set. ACP-tag and MCP-tag fusion proteins labeled with CoA 647 should have an excitation maximum at 660 nm and an emission maximum at 673 nm, and can be imaged with a 650 nm laser.
5. We recommend routinely labeling one well of non-transfected or mock-transfected cells as a negative control.

Troubleshooting for Cellular Labeling

No Labeling
If no labeling is seen, the most likely explanation is that the fusion protein is not expressed. Verify the transfection method to confirm that the cells contain the fusion gene of interest. If this is confirmed, check for expression of the ACP- or MCP-tag fusion protein via Western blot.

Weak Labeling
Weak labeling may be caused by insufficient exposure of the fusion protein to the substrate. Try increasing the concentration of

CoA substrate and/or the incubation time, following the guidelines described above. Alternatively, the protein may be poorly expressed and/or turn over rapidly. If the protein has limited stability in the cell, it may help to analyze the samples immediately after labeling.

High Background

Background fluorescence may be controlled by reducing the concentration of CoA substrate used and by shortening the incubation time. The presence of fetal calf serum or BSA during the labeling incubation should reduce non-specific binding of substrate to surfaces.

Signal Strongly Reduced After Short Time

If the fluorescence signal decreases rapidly, it may be due to instability of the fusion protein. The signal may be stabilized by fixing the cells. Alternatively, try switching the ACP- or MCP-tag from the N-terminus to the C-terminus or vice versa.

Photobleaching is generally not a problem as the CoA 647 substrate is very photostable. However, if problems with photobleaching arise, addition of a commercially available anti-fade reagent may be helpful.