CoA 488

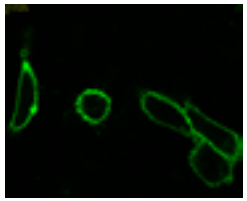
1-800-632-7799
info@neb.com
www.neb.com

S9348S 008130316031

S9348S

50 nmol **Lot: 0081303**

Store at –20°C **Exp: 3/16**



Live CHO-K1 cells transiently transfected with pACP-GPI. Cells were labeled with CoA 488 (green) in the presence of ACP Synthase for 60 minutes.

Introduction

CoA 488 is a photostable fluorescent substrate used to label ACP-tag and MCP-tag fusion proteins exposed on the surface of living cells. This cell-impermeable CoA substrate is based on the ATTO-TEC dye ATTO 488, and is suitable for standard fluorescein filter sets. It has an excitation maximum at 506 nm and an emission maximum at 526 nm. This package contains 50 nmol of CoA 488 substrate, sufficient to make 10 ml of a 5 µM ACP-tag or MCP-tag fusion protein labeling solution.

The ACP-tag and MCP-tag are small polypeptide tags (8 kDa) based on the acyl carrier protein. MCP-tag contains two mutations (D36T and D39G). Both allow the specific, covalent attachment of virtually any molecule to a protein of interest. Substrates are derivatives of coenzyme A (CoA). In the labeling reaction, the substituted phosphopantetheine group of CoA is covalently attached to a conserved serine residue of the ACP-tag or the MCP-tag by a phosphopantetheinyl transferase (SFP Synthase or ACP Synthase).

While ACP Synthase (NEB #9301) will preferentially modify the ACP-tag, SFP Synthase (NEB #P9302) will label both ACP-tag and MCP-tag.

Having no cysteines, the ACP-tag and the MCP-tag are particularly suited for specifically labeling cell-surface proteins, and should be useful for labeling secreted proteins with disulfide bridges such as antibodies.

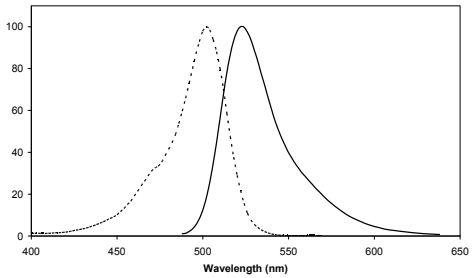


Figure 1. *Excitation (dotted line) and emission spectra of CoA 488 coupled to ACP-tag in buffer at pH 7.4.*

There are two steps to using this system: subcloning and expression of the protein of interest as an ACP-tag or MCP-tag fusion, and labeling of the fusion protein using the appropriate synthase with the CoA substrate of choice. Expression of ACP- and MCP-tagged proteins is described in the documentation supplied with the pACP-tag and pMCP-tag plasmids, respectively. The labeling of the fusion proteins with the CoA substrate is described below.

Materials Required but not Supplied

ACP Synthase (NEB #P9301) for labeling ACP-tag

SFP Synthase (NEB #P9302) for labeling ACP-tag or MCP-tag

Cells expressing ACP-tag or MCP-tag fusion proteins

Tissue culture materials and media

Transfection reagents

Fluorescence microscope with suitable filter set

DMSO

Storage

CoA 488 should be stored at –20°C (long term) or at 4°C in the dark (short term, less than 4 weeks). Protect the substrate from light and moisture. With proper storage at –20°C the substrate should be stable for at least 2 years dry or 3 months dissolved in DMSO.

Quality Controls

Purity and Characterization: Purity of CoA 488 was determined to be 99% by HPLC analysis. Molecular weight $[M]^-$ was determined by MS to be 1477.2 (1477 expected).

***In vitro* Protein Labeling:** Reaction of CoA 488 (10 µM) with purified ACP-MBP (Maltose Binding Protein, 5 µM) and SFP Synthase (1 µM) *in vitro*, followed by mass spec analysis, indicated an efficiency of labeling of ≥ 95%.

Cellular Protein Labeling: Cells transfected with pACP-GPI expressing ACP-GPI (cell surface) were labeled with 5 µM CoA 488 using SFP Synthase for 60 minutes, and visualized by confocal microscopy. Surface target was efficiently labeled.

Instructions for Use

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 for Labeling Reaction

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

These recommendations are for culturing cells in polystyrene plates. For confocal imaging, we recommend using chambered coverglass such as Lab-Tek II Chambered Coverglass which is available in a 1, 2, 4 or 8 well format from Nunc (www.nuncbrand.com).

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 488 should have an excitation maximum at 506 nm and an emission maximum at 526 nm, and can be imaged with standard fluorescein filter sets.
5. We recommend routinely labeling one well of non-transfected or mock-transfected cells as a negative control.

Usage Notes

Optimizing Labeling

Optimal substrate concentrations and reaction times range from 1–10 µM and 30–60 minutes, respectively, depending on experimental conditions and expression levels of the ACP-tag and MCP-tag fusion protein. Best results are usually obtained at concentrations between 1 and 5 µM substrate and 60 minutes reaction time. Increasing substrate concentration and reaction time usually results in a higher background and does not necessarily increase the signal to background ratio.

Stability of Labeling

The turnover and internalization rates of the ACP-tag and MCP-tag fusion protein under investigation may vary widely depending on the fusion partner. Where protein turnover is rapid, we recommend analyzing the cells under the microscope immediately after the labeling reaction or fixing the cells directly after labeling.

Fixation of Cells

After labeling the ACP-tag or MCP-tag fusion proteins, the cells can be fixed with standard fixation methods such as para-formaldehyde, ethanol, methanol, methanol/acetone etc., without loss of signal. We are not aware of any incompatibility of the CoA label with any fixation method.

Counterstaining

Cells can be counterstained with any live-cell dye that is compatible with the fluorescent properties of the substrate for simultaneous microscopic detection. We routinely add 5 µM Hoechst 33342 to the labeling medium as a DNA counterstain for nuclear visualization.

(see other side)

CERTIFICATE OF ANALYSIS

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 488 substrate is very photostable. However, if problems with photobleaching are experienced, addition of a commercially available anti-fade reagent may be helpful.

Instructions for Labeling of Proteins *in vitro*:

1. Dissolve the vial of CoA 488 substrate (50 nmol) in 50 µl of DMSO to yield a stock solution of 1 mM CoA substrate. Mix by vortexing for 10 minutes until all the CoA 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
Deionized Water	28.25 µl	
1 M HEPES	2.5 µl	50 mM
50 mM DTT	1 µl	1 mM
50 mM $MgCl_2$	10 µl	10 mM
50 µM ACP-tag Purified Protein	5 µl	5 µM
40 µM ACP Synthase	1.25 µl	1 µM
250 µM CoA 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 CoA fusion protein by gel filtration or dialysis. Please refer to the vendor's instructions for the separation tools used.

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 ACP- or MCP-tag. The stability of the ACP- or MCP-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.

ACP- or MCP-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 the ACP- or MCP-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 the 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 ACP-tag/MCP-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 the ACP- or MCP-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 ACP- or MCP-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 the fusion protein is particularly sensitive to degradation or to loss of activity, try reducing the labeling time or decreasing the labeling temperature. We recommend overnight incubation when labeling at 4°C.