CoA-SH

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S9352S 004140817081

S9352S

2 mg **Lot: 0041408**

Store at: –20°C **Exp: 8/17**

Introduction

CoA-SH is a maleimide-reactive building block for the one-step synthesis of ACP-tag or MCP-tag substrates from maleimide-containing precursors including fluorophores, peptides or oligonucleotides.

The ACP-tag and MCP-tag are polypeptide tags (8 kD) 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 derivates 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 phosphopantetheine transferase (SFP Synthase or ACP Synthase).

While the ACP Synthase (NEB #P9301) will modify predominantly the ACP-tag, the SFP Synthase (NEB #P9302) will label ACP-tag and MCP-tag. CoA substrates react with both the 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.

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. In this document, the labeling of the fusion proteins with CoA substrates is described. The cloning of ACP-tag and MCP-tag protein fusions is described in the documentation supplied with the ACP-tag or MCP-tag plasmids.

Storage

Store the vials containing the CoA substrate at –20°C. The CoA-SH building block should be dissolved in Tris buffer pH 7.5. Choose a solvent volume that will be compatible with the final use. Unused building block in Tris buffer pH 7.5 should be stored at –20°C under argon.

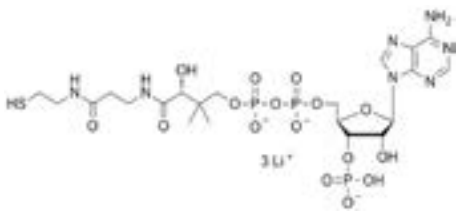

Figure 1. *Structure of CoA-SH (MW 785.3 g/mol)*

Quality Control

Purity and Characterization: Purity of CoA-SH was determined to be 99% by HPLC analysis. Molecular weight $[M]^-$ was determined by MS to be 766.1 (766.1 expected).

Reaction Conditions for Chemical Coupling

CoA-SH is a maleimide-reactive building block which can be used for the one-step synthesis of CoA Sub-strates from maleimide-containing precursors. This building block allows you to make custom CoA substrates for label-ing ACP-tag or MCP-tag fusion proteins for a wide range of applications.

A typical reaction is performed at 30°C overnight by using N,N-dimethylformamide (DMF) and Tris-buffer (pH 7.5) as solvents. Equimolar quantities of CoA-SH and reactive maleimide are recommended.

Example Reaction: Coupling of CoA-SH to a Maleimide-reactive Precursor

Figure 2. *Coupling of CoA-SH to a maleimide-reactive precursor. A solution of a maleimide derivative (1 eq, 6.6 µmol) in DMF (450 µl) was added to a solution of CoA-SH (6.2 mg, 1.2 eq, 7.9 µmol) in Tris-buffer (pH 7.5, 50 µl). The reaction mixture was shaken overnight at 30°C. the solvent was evaporated under vacuum. The solid was dissolved in 1 ml water/acetonitrile (9:1, 0.08% TFA) and purified by HPLC. HPLC fractions containing the product were neutralized with NH_4OH (30% aqueous solution) and lyophilized.*

Protocol for Labeling ACP- or MCP-tag Fusion Proteins with COA-SH Substrates

ACP-tag or MCP-tag fusion proteins can be expressed by transient transfection. For expression of fusion proteins with the ACP-tag or MCP-tag refer to the guidelines with the expression plasmid. For cell culture and transfection methods, refer to established protocols.

Dissolve one vial of CoA substrate (50 nmol) in 50 µl of DMSO to yield a solution of 1 mM CoA substrate in DMSO. Mix for 10 minutes until all the CoA 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 your requirements. The substrate is soluble up to at least 10 mM.

Thaw one vial or aliquot (see below) of ACP Synthase or SFP Synthase and keep on ice. If you will not use all the ACP Synthase or SFP Synthase immediately (makes 5 ml of labeling medium), aliquot the remaining concentrated ACP Synthase or SFP Synthase into single use aliquots and freeze these aliquots at 80°C. It is essential to avoid freeze-thaw cycles with the ACP Synthase or SFP Synthase. Continue immediately with Step 1 below.

Labeling Reaction

1. Dilute the CoA substrate stock solution 1:200 in medium resulting in a labeling medium of 5 µM. For optimal results, add CoA substrate to complete medium, including serum. 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 ACP-Substrate, $MgCl_2$, and ACP Synthase or SFP Synthase than you will consume 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 30 minutes.

3. Wash the cells three times with tissue culture medium with serum.

4. Image the cells using an appropriate filter set. For example, ACP-tag or MCP-tag fusion proteins labeled with CoA-488 should have an excitation maximum at 502 nm and an emission maximum at 522 nm, and can be imaged with standard fluorescein filter sets.

   We recommend routinely labeling one well of non-transfected or mock-transfected cells as a negative control

Notes

Optimizing labeling

The substrate concentration can be varied between 2 and 20 µM depending on the experimental conditions, expression levels of the ACP-tag or MCP-tag fusion protein, and incubation time with the substrate. Best results are usually obtained at concentrations between 5 and 10 µM. An increase of the substrate concentration usually results in a higher background and does not necessarily increase the signal to background ratio.

The incubation time can be varied between 10 and 60 minutes depending on the experimental conditions, expression levels of the ACP-tag fusion protein and substrate concentration. We recommend routine incubation times of 30 minutes. Longer incubation times tend to result in stronger background staining and do not necessarily increase the signal to background ratio.

Stability of labeling

The turnover rates of the ACP-tag or 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, if possible, fixing the cells directly after labeling.

Fixation of cells

The literature shows that after labeling the ACP-tag or MCP-tag fusion proteins, cells can be fixed with para-formaldehyde without loss of signal (1). We are not aware of any incompatibility of the ACP-tag labels with other fixation methods.

Counterstaining

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

Antibody labeling

The literature shows that antibody labeling at the surface of living cells after ACP-tag or MCP-tag labeling is possible (1). Antibody labeling after fixation of the cells should also be possible according to standard protocols without loss of the ACP-tag or MCP-tag signal (see fixation of cells). The fixation conditions should be selected based on experience with the protein of interest. For example some fixation methods destroy epitopes of certain proteins and therefore do not allow antibody staining afterwards.

CERTIFICATE OF ANALYSIS
(see other side)

Experimental conditions that do not allow fetal calf serum

If fetal calf serum has to be omitted due to the experimental setup, the labeling can be done in medium without serum. Higher background levels might be observed because fetal calf serum in the labeling solution reduces the background staining. We recommend reevaluating the dye concentration and incubation time if this is a problem. The addition of 0.5% BSA may be helpful in some cases to block non-specific background.

Troubleshooting

No labeling

If no labeling is seen, there is probably a problem with the expression of your fusion protein. Verify your transfection method to confirm that the cells contain the fusion gene of interest. If this is confirmed, check for expression of the ACP-tag or MCP-tag fusion protein.

Weak labeling

Weak labeling may be caused by insufficient exposure of the fusion protein to the substrate. Try increasing the concentration of CoA substrate, ACP Synthase, or SFP Synthase, and/or the incubation time. Improving the protein expression may also improve the signal. 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. Addition of DNAse I (10 µM/ml final concentration) may also help reducing the background that may be caused by non-transfected plasmid DNA aggregating at the surface of cells.

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.

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

References

1. Yin, J. et al. (2005) *Chemistry & Biology*, 12, 999–1006.
2. George, N. et al. (2004) *JACS*, 126, 8896–8897.
3. Vivero-Pol, L. et al. (2005) *JACS*, 127, 12770-12771.