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FAQs for ACP-tag

GENERAL QUESTIONS

1. What is the ACP-tag?

ACP-tag (77 amino acids) is based on Acyl Carrier Protein.

2. How does it work?

ACP-tag can be labeled with Coenzyme A (CoA) derivatives using ACP Synthase forming a covalent ester bond. The small ACP-tag is particularly suited to selectively label the extracellular portion of membrane proteins.

3. How specific is the binding of substrate to the ACP-tag?

This reaction is highly specific. CoA cannot label ACP-tag in the absence of SFP or ACP synthase.

CLONING AND EXPRESSION

4. What linker type and length would you recommend?

In general, we recommend keeping the linker as short as possible to minimize chances for non-specific protease cleavage of overly long, unstructured peptides. ACP-tag has been cloned as a fusion without a linker present.

5. Can I clone my protein as fusion to the N- or C-terminus of the ACP-tag?

The ACP-tag must be cloned so that it is orientated to the extracellular surface of the plasma membrane so that the tag is accessible to its substrate and synthase.

CELL APPLICATIONS

6. Are ACP-tag substrates stable to fixation?

The fluorescence from ACP-tag substrates is derived from an organic fluorophore. Therefore, labeled ACP-tag fusion proteins are very stable to fixations and do not lose signal intensity in contrast to some GFP spectral variants.

7. Can ACP-tag be multiplexed with other protein labeling systems (GFP, Antibody)?

Yes, ACP-tag has been cloned into a construct containing GFP to compare outside and inside pools of the same protein (Jacquier et al., *PNAS*. 2006, George et al., *JACS*. 2004).

8. Can you use ACP-tag for in vivo FRET?

ACP-tag has been used to label cell surfaces for FRET analysis (Meyer et al., *PNAS*. 2006, Meyer et al., *FEBS*. 2006).

9. Does the ACP-tag labeling reaction work in Yeast?

Yes, if the ACP-tag is cloned so that it is orientated to the extracellular surface of the

cell wall (Vivero-Pol et al., *JACS*. 2005; George et al., *JACS*. 2004).