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FAQs for Cellular Imaging and Analysis

GENERAL QUESTIONS

1. What is the SNAP-tag?

SNAP-tag is a highly engineered version of AGT (alkylguanine DNA alkyltransferase), a DNA repair protein. It has been modified to make it smaller (20 kD), react faster with its benzyl guanine substrates and to remove its affinity for DNA. In mammalian cells, SNAP-tag localizes to the cytoplasm and the nucleus.

2. How does it work?

The SNAP-tag is a protein tag that forms a highly stable, covalent thioether bond with fluorophores or other substituted groups when appended to benzylguanine. This reaction is highly specific: expressed SNAP-tag fusion proteins can be labeled even in the presence of complex protein mixtures such as found in cells or in cleared bacterial lysates.

3. How does SNAP-tag labeling differ from using GFP fusion proteins?

GFP and SNAP-tags are both valuable technologies used to visualize proteins in live cells. GFP is an intrinsically fluorescent protein derived from *Aequorea victoria* while SNAP-tag is derived from hAGT, a human DNA repair protein. In contrast to GFP, the fluorescence of SNAP-tag fusions can be readily turned on with the addition of a variety of fluorescent probes added directly to the culture media. Substituting different fluorophores or other functionality (biotin, magnetic beads, blocking agents) requires no new cloning or expression, merely incubation of the appropriate substrate with cells, cell lysates or recombinant proteins. See the SNAP-tag[®]/CLIP-tag[™] Technologies to GFP table to see a comparison.

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. A linker of approximately 10 amino acids or less is sufficient for SNAP-tag, preferably not much longer. It should also be possible to have no amino acids between the SNAP-tag and its fusion partner.

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

Yes. SNAP- and CLIP-tags can be fused to either the N- or C-terminus of a protein of interest. However, to label surface proteins on the outside of cells using SNAP-, CLIP- the tag must be cloned so that it is oriented to the extracellular surface of the plasma membrane. In this orientation, the tag is accessible to its fluorophore conjugated substrate.

6. What is the smallest peptide and biggest protein you have cloned as SNAP-tag fusions?

Smallest: The SNAP-tag has been expressed as a fusion with various short signal

sequences, such as CaaX (4 amino acids, part of a farnesylation sequence), GPI anchor signal sequence (30 amino acids) or 3x NLS (24 amino acids) and correctly localized to the plasma membrane or to the nucleus as appropriate.

Biggest: SNAP-b-galactosidase (141 kD), enzymatic activity verified by a colorimetric assay.

7. What is the solubility of SNAP-tag in insect and bacterial expression systems?

Our finding is that SNAP-tag is neutral with regard to the solubility of fusion proteins. SNAP-tag on its own is expressed in a soluble form in bacteria and insect cells.

Naturally, the addition of a fusion partner is likely to influence the solubility of the expressed fusion protein.

8. What competent cell strains does NEB suggest for expression in *E. coli*?

For pSNAP-tag (T7) fusions, T7 Express *I*^q Competent *E. coli* (NEB #C3016) or T7 Expressi *lysY/I*^q Competent *E. coli* (NEB #C3013) are recommended.

9. What competent cell *E. coli* strains are suitable for propagating SNAP-tag plasmids?

NEB 10-beta *E. coli* (High Efficiency) (NEB #C3019) is recommended for propagating or subcloning. However, when using a ClaI site, *dam*⁻/*dcm*⁻ Competent *E. coli* (NEB #C2925) is recommended. ClaI is methylation sensitive.

10. Can SNAP-tag fusions be purified and refolded from inclusion bodies?

SNAP-tag fusion protein has been successfully solubilized from inclusion bodies in bacteria and refolded to yield SNAP-tag capable of reacting with its substrate. (Krayl et al., *Anal. Biochemistry*, 2006.)

CELL APPLICATIONS

11. Are substrates toxic to cells?

No toxicity has been noted by proliferation or viability assays when using up to 20 μM substrate for 2 hours. Most of the substrates can be incubated with cells for 24 hours up to a concentration of 20 μM without significant toxicity.

12. How does SNAP-tag affect localization of the fusion partner?

Many proteins have been expressed as SNAP-tag fusion proteins in mammalian cells and the majority exhibit the correct location in the cell.

13. Can I use cell lines which express endogenous AGT?

SNAP-tag is a highly engineered protein based on AGT. No background labeling from AGT has been detected in common cell lines investigated.

14. How stable is the labeled protein in mammalian cells?

The stability of the tagged protein in the cell is dependent upon the stability of protein of interest. Labeled SNAP-tag fusion protein has been detected for up to 2 days in mammalian cells.

15. Are SNAP-tag substrates stable to fixation?

Yes. SNAP-tag substrates are derived from organic fluorophores which are stable to

fixation. Fluorescently-labeled SNAP-tag fusion proteins do not lose signal intensity in contrast to some GFP spectral variants. After labeling the SNAP-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.

16. Can cells expressing SNAP-tag be fixed prior to labeling?

Yes, however the fixative used and washing may need to be optimized.

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

Yes, labeled SNAP-tag fusion proteins are routinely fixed and processed for indirect immunofluorescence.

18. Can you use SNAP-tag for *in vivo* FRET?

in vivo FRET can be carried out between SNAP-tag and an autofluorescent protein or other tag based system.

19. Can cell-impermeable substrates be microinjected into cells, and how is the excess substrate exported?

Yes, SNAP-tag substrates can be microinjected into cells permitting live cellular imaging in the near infrared range by using substrates carrying the appropriate fluorophore. Excess substrate will be transported out. See Keppler A. et al. (2006) *BioTechniques*, 41, 167 for a systematic study.

20. Does the SNAP-tag labeling reaction work in yeast?

BG-biotin has been successfully used in live yeast *S. cerevisiae*, but long incubation was necessary and the percentage of labeling was lower than anticipated. (Keppler A. et al., *Nature Biotechnology* 21, 2003). With genetic modifications disabling some of the MDR transporter proteins from yeast routine labeling of intracellular SNAP-tag in yeast is possible (McMurray, M.A. and Thorner, J. (2008) *Current Biology*, 18, 1203).

21. What happens to the fluorophore upon proteolysis?

We do not see any accumulation of fluorophore in any cell compartment during and after the labeling, thus we assume that all cell permeable fluorophores simply diffuse out of the cells again or are actively transported to the outside of the cell.

IN VITRO APPLICATIONS

22. What conditions are recommended for SNAP-tag labeling *in vitro*?

The SNAP-tag labeling reaction is tolerant of a wide range of buffers. The requirements of the fusion partner should dictate the buffer selected. The following buffer guidelines are recommended: pH between 5.0 and 10.0, monovalent salts (e.g. sodium chloride) between 50 mM, 250 mM and at least 1 mM DTT. Non-ionic detergents can be added to 0.5% v/v if required, but SDS and other ionic detergents should be avoided entirely because they inhibit the activity of the SNAP-tag. Metal chelating reagents (e.g., EDTA and EGTA) also inhibit SNAP-tag activity and should be avoided.

23. What conditions are incompatible with SNAP-tag labeling *in vitro*?

We recommend avoiding the presence of ionic detergents (e.g. SDS) and chelators such as EDTA.

24. Can SNAP-tag fusion proteins be labeled in a cell lysate?

The reaction between SNAP-tag and its substrate is highly specific, therefore labeling can be carried out in lysates.

25. I have a compound that I would like to couple to a BG derivative. Where can I get advice?

NEB has a range of building blocks that can be used to create novel substrates. Products are supplied with instructions for use that offer information concerning chemical coupling (Building Blocks). NEB is happy to answer any specific technical questions.

26. What is the difference between SNAP-tag and ACP-tag?

SNAP-tag can be used for labeling proteins inside of cells, on cell surfaces, in solution and on surfaces. ACP-tag is ideal for cell surface labeling as its substrates are non-cell permeable. (In particular, ACP-tag is ideal for labeling subunits of multimeric membrane proteins such as ion channels.)

27. What is the difference between SNAP- and CLIP-tag?

SNAP-tag and CLIP-tag are both derived from O⁶-alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag recognizes O⁶-labeled benzylguanine substrates while CLIP-tag recognizes O²-labeled benzylcytosine substrates. Each tag transfers the label from the substrate to itself, resulting in specific covalent labeling. In creating the tags, hAGT has been engineered to no longer interact with DNA, but rather with derivatives of the free benzylguanine or benzylcytosine substrates. The tags exhibit no cross-reactivity with one another, enabling researchers to simultaneously label fusion proteins containing SNAP- and CLIP-tags with different fluorophores in live cells.