Cellular Imaging & Analysis

INTRODUCTION TO THE SNAP-TAG® TECHNOLOGY

Update 2015/16

NEW ENGLAND BioLabs® GmbH

Self-Labeling Tag Technology

New England Biolabs offers an innovative technology for studying the function and localization of proteins in living and fixed cells. Covalent protein labeling offers simplicity and versatility to the imaging of mammalian proteins in live cells, as well as the ability to capture proteins *in vitro*. A single genetic construct generates a fusion protein which, when covalently attached to a variety of fluorophores, biotin or beads, provides a powerful tool for studying protein dynamics. In the first system the protein is labeled by a self-labeling fusion protein; SNAP-tag® or CLIP-tag™.

ADVANTAGES

- **Flexible** - Clone and express once, then use with a variety of fluorescent or nonfluorescent substrates
- **Fast** - Easy-to-use protocols
- **Specific** - Very low background staining
- **Precise** - Label is covalently bound under biological conditions in a defined position
- **Non-toxic** - Substrates are non-toxic to living cells
- **Direct covalent labeling** - No antibodies, leaching or drift
- **Selection** - Choose from a broad selection of commercial substrates, optimized for a range of imaging instrumentation

SNAP-tag and CLIP-tag

The SNAP- and CLIP-tag protein labeling systems enable the specific, covalent attachment of virtually any molecule to a protein of interest. There are two steps to using this system: cloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice. The SNAP-tag is a small protein based on human O^6-alkylguanine-DNA-alkyltransferase (hAGT), a DNA repair protein. SNAP-tag substrates are flurophores, biotin or beads conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker (Figure 1). In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag. CLIP-tag is a modified version of SNAP-tag, engineered to react with benzylcytosine rather than benzylguanine derivatives. When used along with SNAP-tag, CLIP-tag enables the orthogonal and complementary labeling of two proteins simultaneously in the same cells.

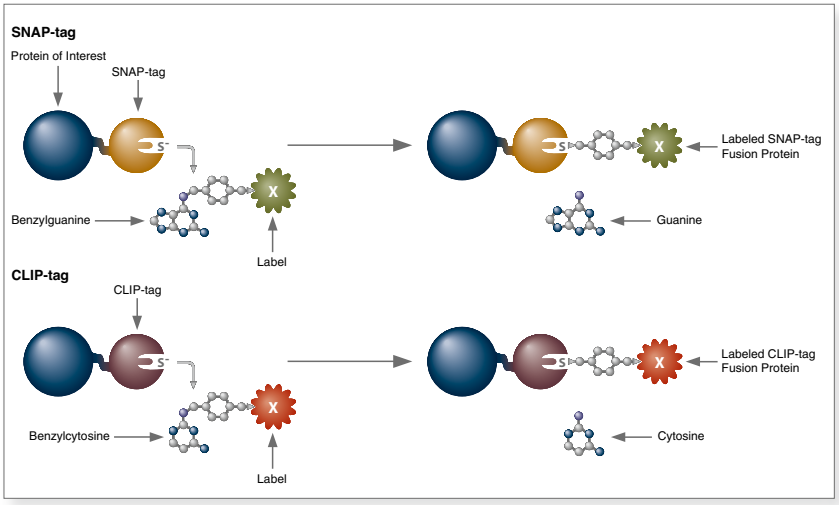
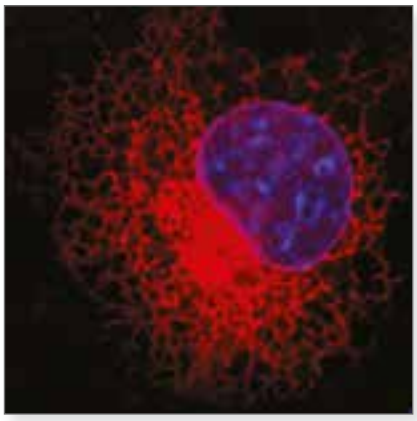


Figure 1: *SNAP-tag (gold) or CLIP-tag (purple) fused to the protein of interest (blue) self-label with label X, releasing either a guanine or a cytosine.*



Live COS-7 cell transiently transfected with pSNAP$_f$-ER. Cells were labeled with SNAP-Cell TMR-Star (red) for 15 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

Enzymatic Labeling Technology

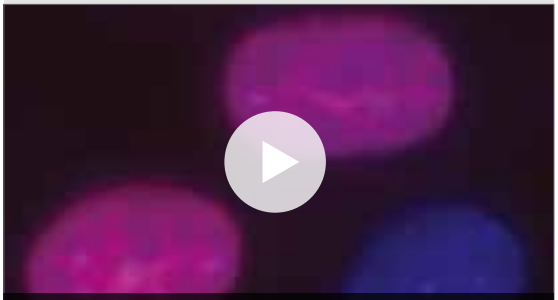
For added flexibility, NEB also offers two systems in which the protein is labeled enzymatically; ACP-tag or MCP-tag. ACP-tag and MCP-tag allow sequential labeling of two different proteins on the surface of living and fixed cells or *in vitro*. In the presence of the appropriate synthase, they can be enzymatically modified with fluorophores, biotin, etc. using substrates that are derivatives of Coenzyme A (CoA). These tags are small and suitable for labeling a variety of proteins including complex cell membrane proteins (e.g., ion channels) which may be sensitive to larger tags.

ACP-tag and MCP-tag

ACP-tag and MCP-tag are small protein tags based on the acyl carrier protein. In contrast to SNAP-tag and CLIP-tag, which are self-labeling, the presence of an added synthase is required for the formation of a covalent link between the ACP-tag or MCP-tag and their substrates, which are derivatives of Coenzyme A. In the labeling reaction, the group conjugated to CoA is covalently attached to the ACP-tag or MCP-tag by a recombinant synthase. Labels can be covalently attached to a tag using either ACP-Synthase (NEB #P9301) for ACP-tag labeling or SFP-Synthase (NEB #P9302) for dual ACP- and MCP-tag labeling.

ADVANTAGES

- **Small** - Expressed tag is only 8 kDa (77 aa)
- **Versatile** - ACP- and MCP-tagged fusions can be co-expressed and sequentially labeled for two color applications on cell surfaces
- **Specific** - Components remain exclusively extracellular, preventing intracellular labeling
- **Precise** - Label is covalently bound under biological conditions in a defined position
- **Non-toxic** - Substrates are non-toxic to living cells
- **Selection** - Choice of substrates available, including 488, 547, 647 nm and biotin



VISIT WWW.NEB.COM/SNAPOVERVIEW FOR AN ONLINE TUTORIAL AND A LABELING PROTOCOL VIDEO.

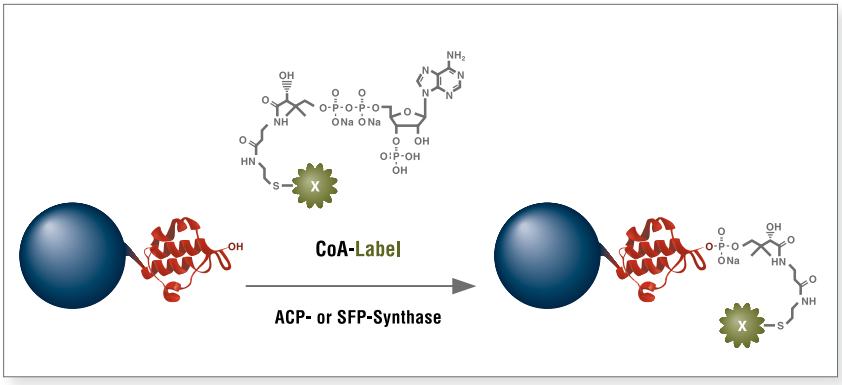
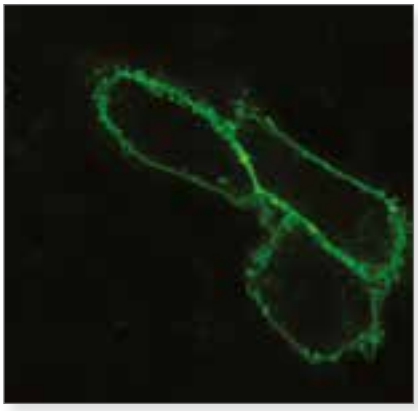


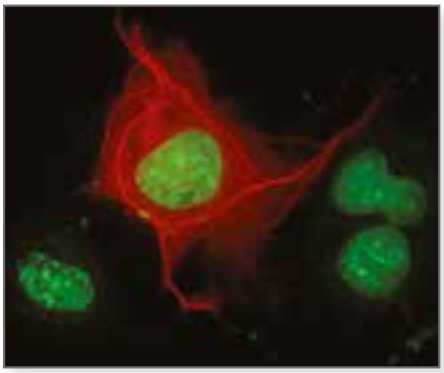
Figure 2: *ACP-tag (red) fused to the protein of interest (blue) is labeled in the presence of a required synthase.*



Live CHO-K1 cells transiently transfected with pACP-GPI. Cells were labeled with CoA-488 (green) in the presence of ACP Synthase for 30 minutes.

Flexibility & Selection

SNAP-tag and CLIP-tag protein labeling systems offer a broad selection of fluorescent substrates optimized for a range of imaging instrumentation. Once cloned and expressed, the tagged protein can be used with a variety of substrates for numerous downstream applications without having to clone again.



Live COS-7 cells transiently transfected with pSNAP$_f$-Cytokeratin13. Cells were labeled with SNAP-Cell TMR-Star (red) for 15 minutes and counterstained with Hoechst 33342 (green pseudocolor) for nuclei.

APPLICATIONS OF SNAP-tag AND CLIP-tag

- Simultaneous dual protein labeling inside or on the surface of live cells
- Protein localization and translocation
- Pulse-chase experiments
- Receptor internalization studies
- Selective cell surface labeling
- Protein pull down assays
- Protein detection in SDS-PAGE
- Flow cytometry
- High throughput binding assays in microtiter plates
- Biosensor interaction experiments
- FRET-based binding assays
- Single-molecule labeling
- Super-resolution microscopy

Comparison of SNAP-tag/CLIP-tag Technologies to GFP

While SNAP-tag/CLIP-tag technologies are complementary to GFP (Green Fluorescent Protein), there are several applications in which SNAP- and CLIP-tag self-labeling approaches may be advantageous.

APPLICATION	SNAP-tag/CLIP-tag	GFP AND OTHER FLUORESCENT PROTEINS
Time-resolved fluorescence	Fluorescence can be initiated upon addition of label	Color is genetically encoded and always expressed. Photoactivatable fluorescent proteins require high intensity laser light, which may activate undesired cellular pathways (e.g., apoptosis)
Pulse-chase analysis	Labeling of newly synthesized proteins can be turned off using available blocking reagents (e.g., SNAP-Cell® Block)	Fluorescence of newly synthesized proteins cannot be specifically quenched to investigate dynamic processes
Ability to change colors	A single construct can be used with different fluorophore substrates to label with multiple colors	Requires separate cloning and expression for each color
Surface specific labeling	Can specifically label subpopulation of target protein expressed on cell surface using non-cell permeant substrates	Surface subpopulation cannot be specifically visualized
Single-molecule detection	Conjugation with high quantum yield and photostable fluorophores	Fluorescent proteins are generally less bright and photobleach quicker than most organic fluorophores
Visualizing fixed cells	Resistant to fixation; strong labeling	Labile to fixation; weak labeling
Pull-down studies	"Bait" proteins can be covalently captured on BG beads	Requires anti-GFP antibody to non-covalently capture "bait" protein, complicating downstream analysis
Live animal imaging	Cell permeable, near-IR dye available, permitting deep tissue visualization	Signal is easily quenched by fixation (whole-mount specimens or thick sections); limited spectral flexibility and weaker fluorescence

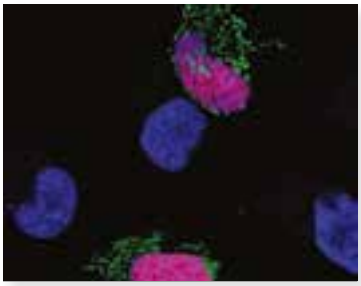
Starter Kits

To introduce these protein labeling technologies, NEB® offers a Starter Kit for each labeling approach that includes a plasmid encoding a tag, a localization control plasmid, two fluorophores and a blocking agent (in most cases). Choose a Starter Kit to become familiar with this easy-to-use technology and build experience with the required techniques.

APPLICATIONS

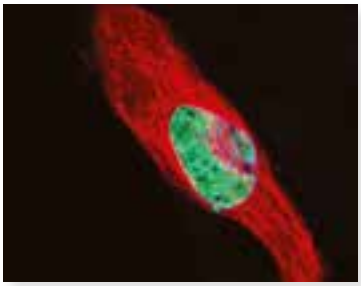
- SNAP-Cell Starter Kit labels intracellular and cell surface proteins with SNAP-Cell 505-Star or SNAP-Cell TMR-Star
- SNAP-Surface Starter Kit labels cell surface proteins with SNAP-Surface 488 or SNAP-Surface 549
- CLIP-Cell Starter Kit labels intracellular and cell surface proteins with CLIP-Cell 505 or CLIP-Cell TMR-Star
- CLIP-Surface™ Starter Kit labels cell surface proteins with CLIP-Surface 488 or CLIP-Surface 547
- ACP-Surface Starter Kit enzymatically labels cell surface proteins with CoA 488 or CoA 547

SNAP-Cell®



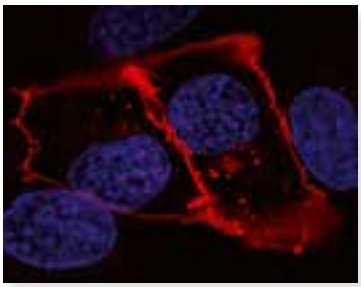
Live U2OS cells transiently co-transfected with pSNAP$_f$-Cox8A (mitochondrial cytochrome oxidase 8A) and pCLIP$_f$-H2B (Histone H2B). Cells were simultaneously labeled with 5 µM SNAP-Cell Oregon Green (green) and 3 µM CLIP-Cell TMR-Star (red) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

CLIP-Cell™



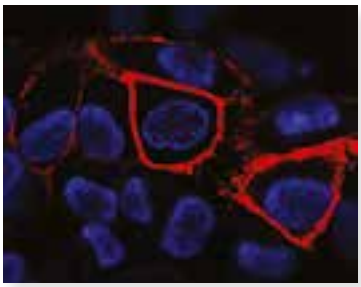
Live HeLa cell transfected with pCLIP$_f$-H2B and pSNAP$_f$-tubulin. Cells were labeled with 5 µM CLIP-Cell 505 (green) and 3 µM SNAP-Cell TMR-Star (red) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

SNAP-Surface®



Live U2OS cells transfected with pSNAP$_f$-ADRβ2 (Beta-2 adrenergic receptor). Cells were labeled with 5 µM SNAP-Surface 549 (red) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

ACP-Surface



Live U2OS cells transiently transfected with pACP-ADRβ2 (Beta-2 adrenergic receptor). Cells were labeled on ice with 5 µM CoA-547 (red) in the presence of ACP Synthase for 30 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

PRODUCT	NEB #	PLASMID	FLUOROPHORE	BLOCK	CONTROL	APPLICATION
SNAP-Cell Starter Kit	E9100S	pSNAP$_f$ Vector	SNAP-Cell 505-Star, SNAP-Cell TMR-Star	SNAP-Cell Block	pSNAP$_f$-Cox8A	• Intracellular labeling • Cell surface labeling • *in vitro* analysis
SNAP-Surface Starter Kit	E9120S	pSNAP$_f$ Vector	SNAP-Surface 488, SNAP-Surface 549	SNAP-Surface Block	pSNAP$_f$-ADRβ2	• Cell surface labeling • *in vitro* analysis
CLIP-Cell Starter Kit	E9200S	pCLIP$_f$ Vector	CLIP-Cell 505, CLIP-Cell TMR-Star	CLIP-Cell Block	pCLIP$_f$-H2B	• Intracellular labeling • Cell surface labeling • *in vitro* analysis
CLIP-Surface Starter Kit	E9230S	pCLIP$_f$ Vector	CLIP-Surface 488, CLIP-Surface 547	CLIP-Cell Block	pCLIP$_f$-NK1R	• Cell surface labeling • *in vitro* analysis
ACP-Surface Starter Kit	E9300S	pACP-tag(m)-2 Vector	CoA 488, CoA 547	N/A	pACP-ADRβ2	• Cell surface labeling • *in-vitro* analysis

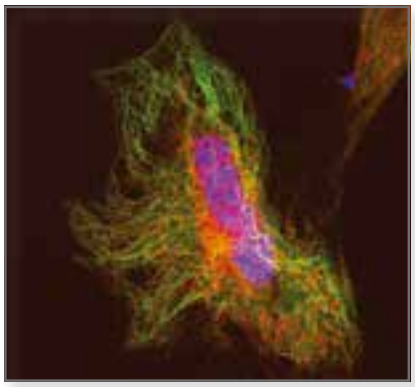
Fluorescent Substrates

NEB offers a large selection of fluorescent labels (substrates) for SNAP-, CLIP-, ACP- and MCP-tag fusion proteins. Cell-permeable substrates (SNAP- and CLIP-Cell) are suitable for both intracellular and cell-surface labeling, whereas non-cell-permeable substrates (SNAP- and CLIP-Surface) are specific for fusion proteins expressed on the cell surface only. ACP-tag/MCP-tag labels require a separate enzyme (ACP or SFP Synthase) for labeling. The labeling reaction is specific for fusion proteins expressed on the cell surface. The same substrates are used to label both the ACP and the MCP tags; specificity is determined by which synthase is used for labeling.

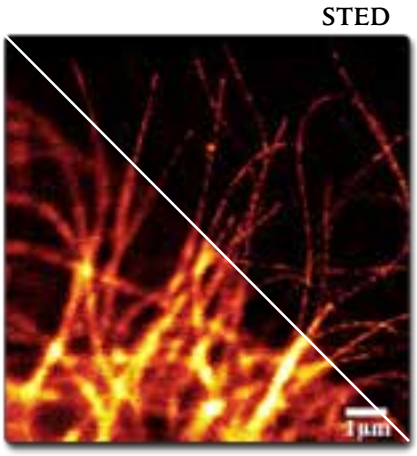
SELF-LABELING TAG					
	APPLICATIONS	NEB #	EXCITATION*	EMISSION*†	SIZE
SNAP-tag	Cell-Permeable				
	SNAP-Cell 360	S9101S	357	437	50 nmol
	SNAP-Cell 430	S9109S	421	444,484	50 nmol
	SNAP-Cell 505-Star	S9103S	504	532	50 nmol
	SNAP-Cell Oregon Green®	S9104S	490	514	50 nmol
	SNAP-Cell Fluorescein	S9107S	500	532	50 nmol
	SNAP-Cell TMR-Star	S9105S	554	580	30 nmol
	SNAP-Cell 647-SiR	S9102S	645	661	30 nmol
	Non-cell-permeable				
	SNAP-Surface Alexa Fluor® 488	S9129S	496	520	50 nmol
	SNAP-Surface 488	S9124S	506	526	50 nmol
	SNAP-Surface Alexa Fluor 546	S9132S	558	574	50 nmol
	SNAP-Surface 549	S9112S	560	575	50 nmol
	SNAP-Surface 594	S9134S	606	626	50 nmol
	SNAP-Surface Alexa Fluor 647	S9136S	652	670	50 nmol
	SNAP-Surface 649	S9159S	655	676	50 nmol
CLIP-tag	APPLICATIONS	NEB #	EXCITATION*	EMISSION*†	SIZE
	Cell-Permeable				
	CLIP-Cell 505	S9217S	504	532	50 nmol
	CLIP-Cell TMR-Star	S9219S	554	580	30 nmol
	Non-cell-permeable				
	CLIP-Surface 488	S9232S	506	526	50 nmol
	CLIP-Surface 547	S9233S	554	568	50 nmol
	CLIP-Surface 647	S9234S	660	673	50 nmol
ENZYMATIC TAG					
	APPLICATIONS	NEB #	EXCITATION*	EMISSION*†	SIZE
ACP-/MCP-tag	Non-cell-permeable				
	CoA 488	S9348S	506	526	50 nmol
	CoA 547	S9349S	554	568	50 nmol
	CoA 647	S9350S	660	673	50 nmol

* Excitation and emission values determined experimentally for labeled protein tag.
† Colors are based on the electromagnetic spectrum. Actual color visualization may vary.

This table lists all currently available fluorescent substrates for SNAP-, CLIP-, ACP- and MCP-tags, along with excitation and emission wavelengths (determined from a labeled fusion tag, rather than the unreacted substrate).



Live HeLa cell transfected with pSNAP$_f$-ER (endoplasmic reticulum) and pCLIP$_f$-tubulin. Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (#S9217; green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

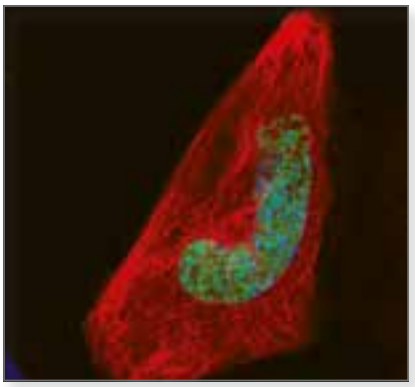


Composite image in confocal (lower left) and super resolution (upper right) showing live U2-OS cells expressing a centrosomal fusion protein, Cep41-SNAP, labeled with SNAP-Cell 647-SiR (#S9102). Scale bar is 1 µm.

Cloning Vectors & Control Plasmids

Cloning vectors are available for SNAP-tag and CLIP-tag fusion protein expression in mammalian and bacterial systems.

PRODUCT	NEB #	FEATURES	SIZE
pSNAP$_f$ Vector	N9183S	stable and transient mammalian expression	20 µg
pSNAP-tag(T7)-2 Vector	N9181S	bacterial expression under T7 control	20 µg
pCLIP$_f$ Vector	N9215S	stable and transient mammalian expression	20 µg



Live HeLa cell transfected with pSNAP$_f$-tubulin and pCLIP$_f$-H2B constructs generated using pSNAP$_f$ and pCLIP$_f$ vectors. Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

Companion Products

Biotin Labels

For optimal flexibility with existing technologies, biotinylated labels are available for studies using streptavidin platforms. Cell-permeable (SNAP-Biotin and CLIP-Biotin) or non-cell-permeable substrates (CoA-Biotin) are suitable for applications such as biotinylation of fusion proteins in or on living cells for detection with streptavidin fluorophore conjugates or labeling in solution for analysis by SDS-PAGE/Western blot. Biotin labels are also used for binding and protein interaction studies.

PRODUCT	NEB #	SIZE
SNAP-Biotin®	S9110S	50 nmol
CLIP-Biotin	S9221S	50 nmol
CoA-Biotin	S9351S	50 nmol

Vista Label

SNAP-Vista Green fluorescent label can be used to label SNAP-tag fusions in cell lysates or as purified proteins for detection by SDS-PAGE. The substrates are optimal for visualization using laser based gel scanners.

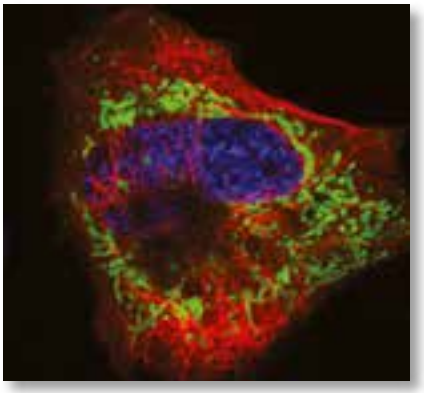
PRODUCT	NEB #	SIZE	EXCITATION	EMISSION
SNAP-Vista® Green	S9147S	50 nmol	500	524

Companion Products (continued)

Blocking Agents

Blocking agents are non-fluorescent compounds that block the reactivity of the SNAP- or CLIP-tag intracellularly (SNAP-Cell™ Block and CLIP-Cell™ Block) or on the surface of cells (SNAP-Surface™ Block and CLIP-Cell™ Block). They can be used to generate inactive controls in live and fixed cell labeling experiments performed with SNAP- or CLIP-tag fusion proteins. Their irreversible blocking makes them ideal for pulse-chase applications.

PRODUCT	NEB #	APPLICATION	SIZE
SNAP-Cell Block	S9106S	Block SNAP-tag inside live cells and *in vitro*	100 nmol
CLIP-Cell Block	S9220S	Block CLIP-tag inside or on the surface of live cells and *in vitro*	100 nmol
SNAP-Surface Block	S9143S	Block SNAP-tag on the surface of live cells and *in vitro*	200 nmol



Live HeLa cell transfected with pSNAP$_f$-tubulin and pCLIP$_f$-Cox8A (mitochondrial cytochrome oxidase 8A). Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

SNAP-Capture

SNAP-Capture products are magnetic or non-magnetic agarose beads coupled to a benzylguanine substrate, used to selectively capture and immobilize SNAP-tag fusion proteins from solution. These beads have a high loading capacity for SNAP-tag fusion proteins and show very low non-specific adsorption of proteins from a complex lysate, making them suitable for pull-down applications.

PRODUCT	NEB #	SIZE
SNAP-Capture Pull-Down Resin	S9144S	2 ml
SNAP-Capture Magnetic Beads	S9145S	2 ml

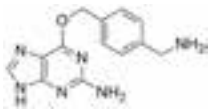
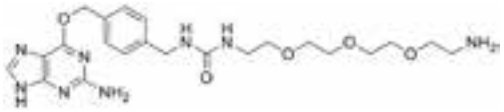
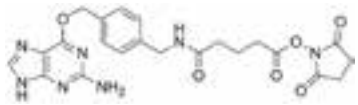
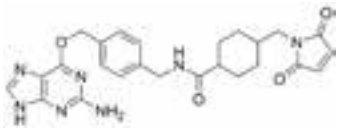
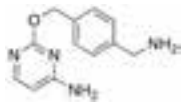
Synthases

ACP Synthase catalyzes the covalent transfer of substituents from derivatized coenzyme A (CoA) substrates to ACP-tagged fusion proteins exposed on the surface of living or fixed cells. SFP Synthase catalyzes the covalent transfer of substituents from derivatized coenzyme A (CoA) substrates to ACP- or MCP-tagged fusion proteins exposed on the surface of living or fixed cells.

PRODUCT	NEB #	SIZE
ACP Synthase	P9301S	25 nmol
SFP Synthase	P9302S	25 nmol

Building Blocks

For advanced users with novel probes interested in working with SNAP-tag and CLIP-tag technologies, a complete line of building blocks is available for linkage of the core benzylguanine (BG) and benzylcytosine (BC) moieties to activated esters, primary amines and thiol groups. The variety of functional groups allows a choice of chemical coupling approaches to suit the molecule or surface to be coupled for the generation of custom substrates.

PRODUCT	NEB #	STRUCTURE	APPLICATION	SIZE
BG-NH2	S9148S		SNAP-tag substrate. Suitable for linkage to NHS esters and other activated carboxylic esters.	2 mg
BG-PEG-NH2	S9150S		SNAP-tag substrate. PEG-linker gives superior flexibility. Particularly suited for immobilization on solid surfaces.	2 mg
BG-GLA-NHS	S9151S		SNAP-tag substrate. Activated as NHS ester. Reacts with primary amines.	2 mg
BG-Maleimide	S9153S		SNAP-tag substrate. Activated as maleimide. Reacts with thiols.	2 mg
BC-NH2	S9236S		CLIP-tag substrate. Suitable for linkage to NHS esters and other activated carboxylic esters.	2 mg

Purified Protein

Purified protein can be used as a positive control for *in vitro* labeling with SNAP-tag fluorescent substrates.

PRODUCT	NEB #	SIZE	CONCENTRATION	MOLECULAR WEIGHT
SNAP-tag Purified Protein	P9312S	50 µg	50 µM	19,694

Antibodies

The Anti-SNAP-tag Antibody (Polyclonal) can be used in Western blots with SNAP-tag and CLIP-tag proteins. Polyclonal antibodies are produced from the immunization of rabbit with purified recombinant SNAP-tag protein and affinity purified using SNAP-Capture resin.

PRODUCT	NEB #	SIZE
Anti-SNAP-tag Antibody (Polyclonal)	P9310S	100 µl

Troubleshooting Guide

Labeling with SNAP-tag Technology

APPLICATION	PROBLEM	CAUSE	SOLUTION
Cellular Labeling	No labeling	Fusion protein not expressed	• Verify transfection • Check expression of fusion protein via Western blot or SDS-PAGE with Vista Green label
	Weak labeling	Poor expression and/or insufficient exposure of fusion protein to substrate	• Increase substrate concentration • Increase incubation time
		Rapid turnover of fusion protein	• Analyze samples immediately or fix cells directly after labeling • Label at lower temperature (4°C or 16°C)
	High background	Non-specific binding of substrates	• Reduce substrate concentration and/or incubation time • Allow final wash step to proceed for up to 2 hours • Include fetal calf serum or BSA during labeling
	Signal strongly reduced after short time	Instability of fusion protein	• Fix cells • Switch tag from N-terminus to C-terminus or vice versa
		Photobleaching	• Add commercially available anti-fade reagent • Reduce illumination time and/or intensity
Labeling in Solution	Precipitation	Insoluble fusion	• Test from pH 5.0 to 10.0 • Optimize salt concentration [50 to 250 mM] • Add 0.05 to 0.1% Tween 20
	Weak or no labeling	Exhaustive labeling has not been achieved	• Increase incubation time to 2 hrs at 25°C or 24 hrs at 4°C • Reduce the volume of protein solution labeled • Check expression of fusion protein via SDS-PAGE with Vista Green label
	Loss of activity	Instability of fusion protein	• Reduce labeling time • Decrease labeling temperature (4°C or 16°C)

FAQs

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

GFP and SNAP-tag 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 functionalities (biotin, magnetic beads, blocking agents) requires no new cloning or expression, merely incubation of the appropriate substrate with cells, cell lysates or recombinant proteins.

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

SNAP-tag and CLIP-tag are both derived from O^6 -alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag recognizes O^6-labeled benzylguanine substrates while CLIP-tag recognizes O^2-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.

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 the SNAP-tag or CLIP-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.

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.

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.

ADDITIONAL FAQS CAN BE FOUND AT WWW.NEB.COM

Reviews:

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Kohl, J. et al. (2014) Ultrafast tissue staining with chemical tags. *Proc. Natl. Acad. Sci. USA,* 111, E3805–E3814.

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