

Cellular Imaging & Analysis

INTRODUCTION TO THE SNAP-TAG[®] TECHNOLOGY



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Self-Labeling Tag Technology

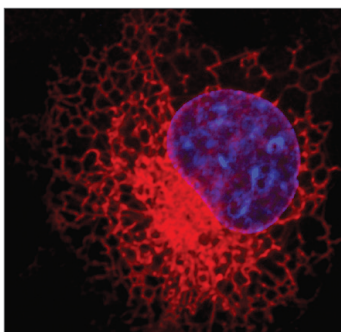
New England Biolabs offers an innovative technology for studying the function and localization of proteins in living and fixed cells. Covalent protein labeling offers simplicity and versatility to the imaging of mammalian proteins in live cells, as well as the ability to capture proteins *in vitro*. 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 this system the protein is labeled by a self-labeling fusion protein; SNAP-tag® or CLIP-tag®.

SNAP-tag and CLIP-tag

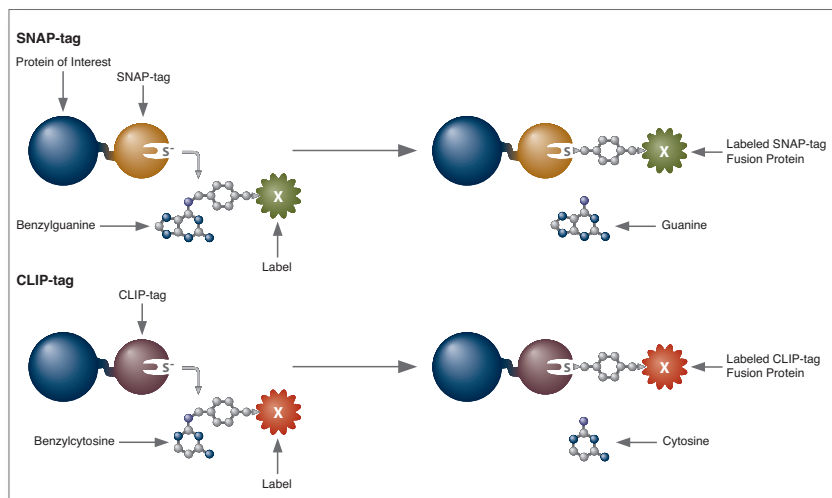
The SNAP- and CLIP-tag protein labeling systems enable the specific, covalent attachment of virtually any molecule to a protein of interest. There are two steps to using this system: cloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice. The SNAP-tag is a small protein based on human O⁶-alkylguanine-DNA-alkyltransferase (hAGT), a DNA repair protein. SNAP-tag substrates are fluorophores, biotin, or beads conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker. 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.

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

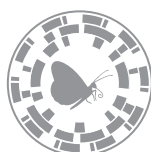


Live COS-7 cell transiently transfected with pSNAP-ER. Cells were labeled with SNAP-Cell TMR-Star (red) for 15 minutes and counterstained with Hoechst 33342 (blue) for nuclei.



Protein labeling with SNAP-tag (gold) and CLIP-tag (purple). The SNAP- or CLIP-tag is fused to the protein of interest (blue). Labeling occurs through covalent attachment to the tag, releasing either a guanine or a cytosine moiety.

DOWNLOAD THE NEB AR APP*



Find an overview of SNAP-tag labeling.

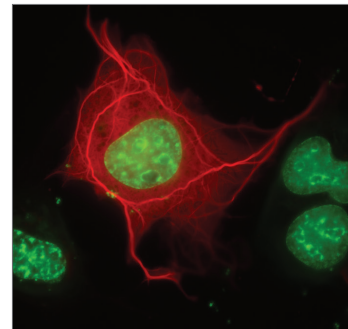
*see back cover for details

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.

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



Live COS-7 cells transiently transfected with pSNAP₇-Cytokeratin13. Cells were labeled with SNAP-Cell TMR-Star (red) for 15 minutes and counterstained with Hoechst 33342 (green pseudocolor) for nuclei.

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 |

Cloning Vectors

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

| PRODUCT | NEB # | FEATURES | SIZE |
|---------------------------|--------|---|-------|
| pSNAP ₇ Vector | N9183S | stable and transient mammalian expression | 20 µg |
| pSNAP-tag(T7)-2 Vector | N9181S | bacterial expression under T7 control | 20 µg |
| pCLIP ₇ Vector | N9215S | stable and transient mammalian expression | 20 µg |

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-BG resin.

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

Fluorescent Substrates

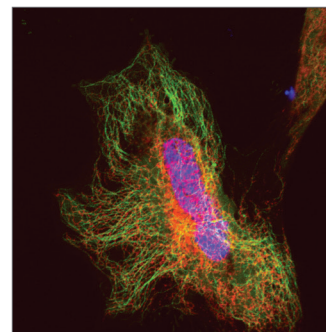
NEB offers a large selection of fluorescent labels (substrates) for SNAP-tag and CLIP-tag fusion proteins. Cell-permeable substrates (SNAP- and CLIP-Cell) are suitable for both intracellular and cell-surface labeling, whereas non-cell-permeable substrates (SNAP- and CLIP-Surface) are specific for fusion proteins expressed on the cell surface only. The labeling reaction is specific for fusion proteins expressed on the cell surface.

| SELF-LABELING TAG | | | | | |
|-------------------------------|---------------------------|-----------------------|-------------|------------|---------|
| | APPLICATIONS | NEB # | EXCITATION* | EMISSION** | SIZE |
| | SNAP-tag | Cell-Permeable | | | |
| 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 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 | 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 |

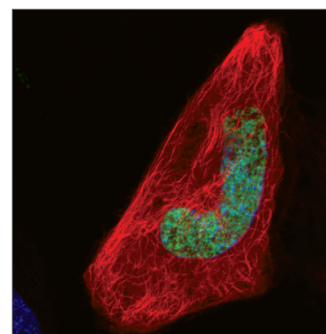
* Excitation and emission values determined experimentally for labeled protein tag.

** Colors are based on the electromagnetic spectrum. Actual color visualization may vary.

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



Live HeLa cell transfected with pSNAP_i-ER (endoplasmic reticulum) and pCLIP_i-tubulin. 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.



Live HeLa cell transfected with pSNAP_i-tubulin and pCLIP_i-H2B constructs generated using pSNAP_i and pCLIP_i 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.

Biotin Labels

For optimal flexibility with existing technologies, biotinylated labels are available for studies using streptavidin platforms. Cell-permeant (SNAP-Biotin and CLIP-Biotin) substrates are suitable for applications such as biotinylation of fusion proteins 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 |

Purified Protein

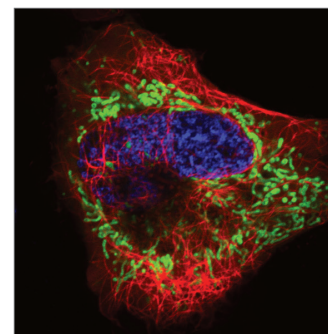
Purified protein can be used as a positive control for *in vitro* labeling with various SNAP-tag fluorescent substrates.

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

Blocking Agents

Blocking agents are non-fluorescent substrates 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 or fixed cell and *in vitro* 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 <i>in vitro</i> | 100 nmol |
| CLIP-Cell Block | S9220S | Block CLIP-tag inside or on the surface of live cells and <i>in vitro</i> | 100 nmol |
| SNAP-Surface Block | S9143S | Block SNAP-tag on the surface of live cells and <i>in vitro</i> | 200 nmol |

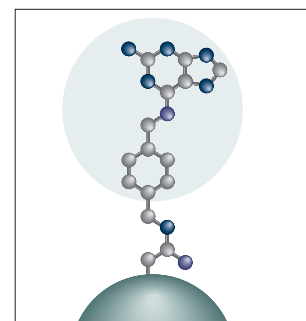


Live HeLa cell transfected with pSNAP₇-tubulin and pCLIP₇-Cox8A (mitochondrial cytochrome oxidase 8A). Cells were labeled with 3 μM SNAP-Cell TMR-Star (red) and 5 μM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

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.

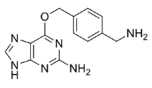
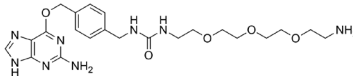
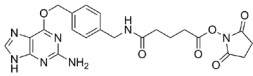
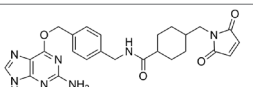
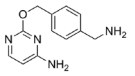
| PRODUCT | NEB # | SIZE |
|-------------------------------------|--------|------|
| SNAP-Capture Pull-Down Resin | S9144S | 2 ml |
| SNAP-Capture Magnetic Beads | S9145S | 2 ml |



Substrate structure on SNAP-Capture Pull-Down Resin

Building Blocks

For advanced users with novel probes interested in working with SNAP-tag and CLIP-tag labeling 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 |



Troubleshooting Guide: Labeling with SNAP-tag Technology

| APPLICATION | PROBLEM | CAUSE | SOLUTION |
|----------------------|--|---|--|
| Cellular Labeling | No labeling | Fusion protein not expressed | <ul style="list-style-type: none"> • Verify transfection • Check expression of fusion protein via Western blot or SDS-PAGE with fluorescent substrate. |
| | Weak labeling | Poor expression and/or insufficient exposure of fusion protein to substrate | <ul style="list-style-type: none"> • Increase substrate concentration • Increase incubation time |
| | | Rapid turnover of fusion protein | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • Fix cells • Switch tag from N-terminus to C-terminus or vice versa |
| Photobleaching | | <ul style="list-style-type: none"> • Add commercially available anti-fade reagent • Reduce illumination time and/or intensity | |
| Labeling in Solution | Precipitation | Insoluble fusion | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • 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 fluorescent substrate. |
| | Loss of activity | Instability of fusion protein | <ul style="list-style-type: none"> • Reduce labeling time • Decrease labeling temperature (4°C or 16°C) |

FAQs

How does SNAP-tag labeling differ from using GFP 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 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.

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

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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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