Simultaneous Fluorescent Labeling of Proteins in Living Cells

Introduction

Specific labeling of proteins via self-labeling SNAP-tag® and CLIP-tag™ provides an innovative tool for studying the function and localization of proteins in live and fixed cells (1,2). Covalent protein labeling brings simplicity and versatility to the imaging of mammalian proteins in living cells, as well as the ability to capture proteins *in vitro*. The creation of 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 investigating proteins via fluorescent imaging, pull-down, and other biochemical analyses. Unlike some commonly used autofluorescent proteins, the fluorescent signal from a self-labeling tag can be initiated upon addition of a label, allowing time-resolved studies of protein expression, localization and degradation. A collection of non-fluorescent substrates that block SNAP- and CLIP-tag reactivity enables pulse-chase studies and assessment of the temporal dynamics of nascent protein synthesis and complex formation in live cells (3).

General Protocol

1. Seed trypsinized U2OS cells in an 8-well Lab-Tek II Chambered Coverglass (Nalgene #155409).
2. Co-transfect the cells with 0.3 µg each of $SNAP_f$ and $CLIP_f$ fusion protein construct and incubate samples for 18-24 hours at 37°C, 5% CO_2.
3. Remove transfection complex media, wash cells twice with complete media, and label cells with 3 µM SNAP-Cell® TMR-Star (NEB #S9105) and 5 µM CLIP-Cell™ 505 (NEB #S9217) labeling media for 30 minutes at 37°C, 5% CO_2.
4. Remove the labeling media and add media containing 5 µM Hoechst 33342 to the cells for 2-3 minutes for nuclear counterstaining (optional).
5. Wash cells 3X with complete media then incubate the samples for 30 minutes at 37°C, 5% CO_2 to allow unincorporated substrate to diffuse out of the cells.
6. Replace media one last time and proceeded to imaging.

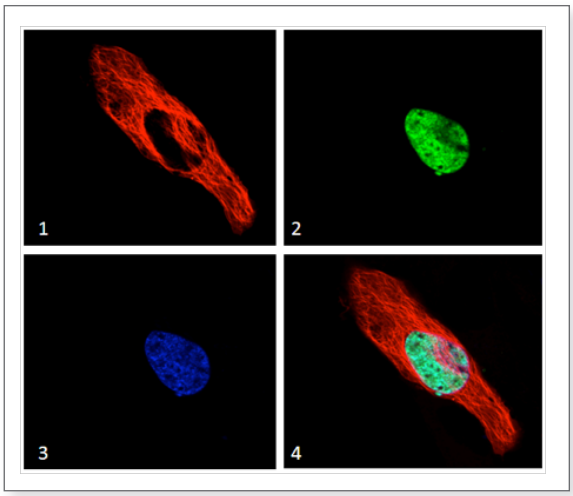

Figure 1. *Orthogonal labeling of $SNAP_f$-tubulin and $CLIP_f$-H2B fusion proteins transiently expressed in live U2OS cells. Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 30 minutes and counterstained with Hoescht 33342 (blue) for nuclei.*

Materials

- $pCLIP_f$-H2B Control Plasmid (NEB #N9218)
- $pSNAP_f$-tubulin (not a catalog product)
- CLIP-Cell 505 (NEB #S9217)
- SNAP-Cell TMR-Star (NEB #S9105)
- U2OS or HeLa cells
- DMEM medium
- Hoechst 33342
- Lab-Tek II Chambered Coverglass (Nalgene)

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Results

SNAP$_f$ and CLIP$_f$ tags for Fluorescent Labeling

SNAP$_f$ and CLIP$_f$ are improved versions of SNAP- and CLIP-tags with increased reaction rates for their fluorescent substrates (4,5). Simultaneous dual labeling of SNAP$_f$ and CLIP$_f$ fusion proteins provides researchers with a unique tool to study proteins with rapid dynamics or fast turnover rates in living cells (3). Figure 1 shows the orthogonal labeling of two proteins in live cells: SNAP$_f$-Tubulin, (a dynamic protein that polymerizes into microtubules), and CLIP$_f$-H2B (histone H2B, a nuclear protein involved in chromatin structure). Figure 2 shows the orthogonal labeling in live HeLa cells transfected with pSNAP$_f$-tubulin and pCLIP$_f$-Cox8A (mitochondrial cytochrome oxidase 8A). The data clearly demonstrate specific labeling of the appropriate target proteins, confirming that SNAP$_f$ and CLIP$_f$ can be used for orthogonal protein labeling in living cells. The fluorescent labeling can be performed simultaneously or sequentially, depending on the experimental needs.

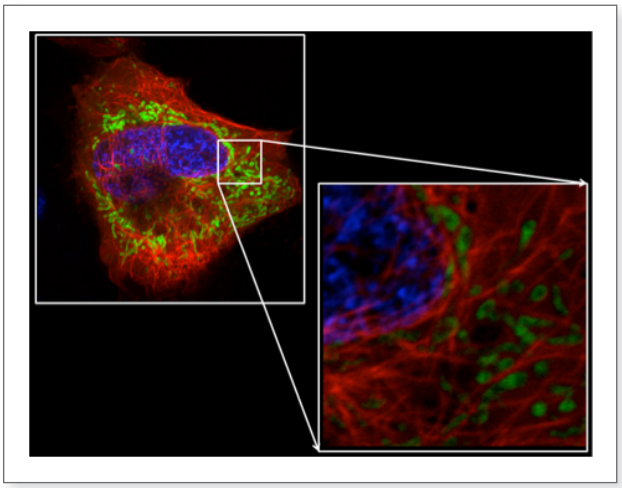

Figure 2. *Orthogonal labeling of SNAP$_f$-Tubulin and CLIP$_f$-Cox8A fusion proteins transiently expressed in live HeLa cells. Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 30 minutes and counterstained with Hoescht 33342 (blue) for nuclei.*

Summary

Live cell imaging using fluorescent tags has been widely used by researchers to study protein expression and location. Its applications include assessing protein dynamics, cellular structures, and organogenesis.

CLIP$_f$- and SNAP$_f$-tag are valuable tools for the selective labeling of proteins in live cells. As both tags have been engineered to react with distinct classes of substrates, the system can simultaneously visualize two proteins in the same cellular environment. The dynamics of their interaction, localization and stability can be readily examined following a variety of cellular stimuli.

References:
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