

Study of Mouse Tumor Models with an IRDye 800CW SNAP-tag Conjugate

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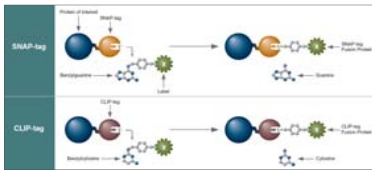


Abstract

Non-invasive optical analysis of molecular targets inside living animals has become an important tool for disease progression and treatment assessment. One of the essential elements of molecular imaging is the development of specific, sensitive imaging contrast agents to investigate these biological processes. The use of longer wavelength dyes, i.e. near-infrared (NIR) dyes, presents distinct advantages over inherently fluorescent protein tags, including reduced autofluorescence background and increased sensitivity in imaging small animals. In addition, the adoption of efficient and specific labeling techniques is a key step in the generation of protein-based fluorescent imaging agents. In the present study, we explore the versatility of a self-labeling protein termed SNAP-tag, derived from human O⁶-alkylguanine-DNA alkyltransferase, in tagging polypeptides with a near-infrared dye, IRDye[®] 800CW. In cell-based assays, an IRDye 800CW conjugated SNAP-tag successfully transfected cells transiently transfected with a pSNAP-ADRB2 plasmid. For *in vivo* experiments, a synthetic epidermal growth factor receptor (EGFR)-binding ligand was used to construct an IRDye 800CW-EGFR-SNAP-tag. Following injection of the agent into nude mice bearing EGFR-overexpressing A431 xenografts, tumors were clearly visualized with a Pearl[™] Imaging system. Furthermore, the specificity of binding was demonstrated in competition experiments with unlabeled EGF. Similar probes labeled with visible fluorophores were used to image EGFR-expressing cultured cells by confocal microscopy. This fluorescent imaging system using a self-labeling protein tag and highly sensitive organic dyes provides versatile tools for cancer research, drug discovery and small animal imaging.

SNAP-tag Technologies

SNAP-tag and CLIP-tag react with O⁶-benzylguanine (BG) and O⁶-benzylcytosine (BC) conjugates, respectively (Figure 1). Each tag reacts only once with a single substrate molecule, with a well defined mechanism, predictable stoichiometry and rapid kinetics, irrespective of the protein attached to the tag. A stable thioether bond is formed between the reactive cysteine of the tag and the label.



Mechanism of SNAP-tag (top) and CLIP-tag (bottom) self-labeling reactions.

Table 1. SNAP-tag and CLIP-tag substrates. Cell permeable SNAP-Cell and CLIP-Cell labels are used for labeling total protein pools whereas cell impermeable SNAP-Surface and CLIP-Surface products enable specific labeling of surface localized proteins. Non-fluorescent substrates (Block) that react with the existing SNAP-tag (or CLIP-tag) protein pool enable pulse-chase studies and an examination of the temporal dynamics of nascent protein synthesis in living cells. Near-infrared dyes, such as Surface 782 and IRDye 800CW (LI-COR Biosciences) conjugated to BG are applicable for sensitive detection in cell assays and small animal imaging. Additional products include fluorescent labels (SNAP-Vista and CLIP-Vista) for rapid in-gel-detection, biotin conjugates and a resin with immobilized BG (SNAP-Capture) for protein pull down studies.

Product Type
Starter Kit
Surface 426
Surface:Alexa Fluor [®] 488
Surface 488
Surface 532
Surface:Alexa Fluor [®] 568
Surface 547
Surface 549
Surface 600
Surface 632
Surface:Alexa Fluor [®] 647
Surface 647
Surface 682
Surface 732
Surface 747
Surface 782
Surface Block
Starter Kit
Cell 360
Cell 426
Cell 555
Cell Oregon Green [®]
Cell Fluorescein
Cell TMR-Star
Cell Block

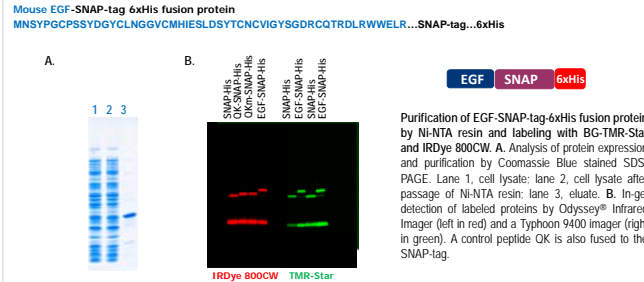
Near-Infrared Imaging of Small Animals

- Light absorption by hemoglobin and other compounds in living tissue limits the depths of tissue penetration.
- Above 900 nm, light absorption by water causes interference.
- In the near-infrared (NIR) region (700-900 nm) the absorption coefficient of tissue is at its lowest and light can penetrate to depths of several centimeters.
- Low autofluorescence of cells and tissues at NIR wavelengths reduce false positives.
- Infrared dyes, such as IRDye 800CW (LI-COR Biosciences) have been widely used for cell assays and small animal imaging.

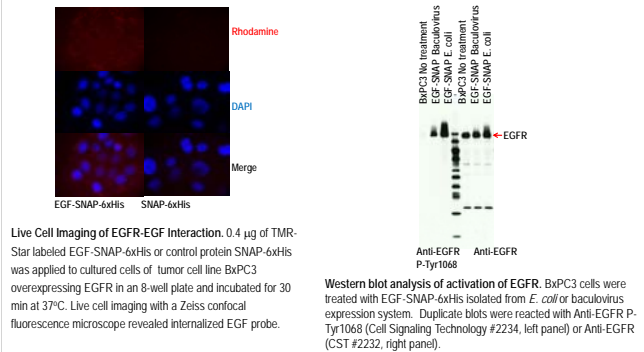
Study of Tumor Models Using SNAP-tag

Epidermal growth factor (EGF) receptor expression is elevated over normal cellular levels in many tumor cell types (e.g. 100-fold over expression in the A431 tumor model). Targeting a tumor-enriched cell surface receptor (e.g. EGFR) with a ligand-conjugated fluorescent probe permits monitoring tumor progression by non-invasive imaging of live whole animals. In this study an imaging probe was generated by conjugation of purified EGF-SNAP-tag with IRDye 800CW and injected into nude mice bearing EGFR-overexpressing A431 xenografts followed by NIR imaging with a Pearl[™] Imaging system.

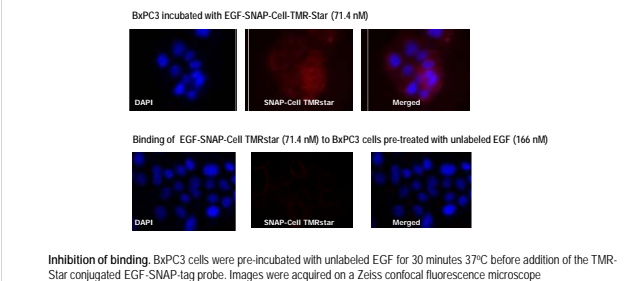
Production of Imaging Probe



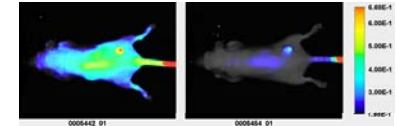
EGF Binding, Internalization and Activation of EGFR



Cell Assay of Binding Specificity

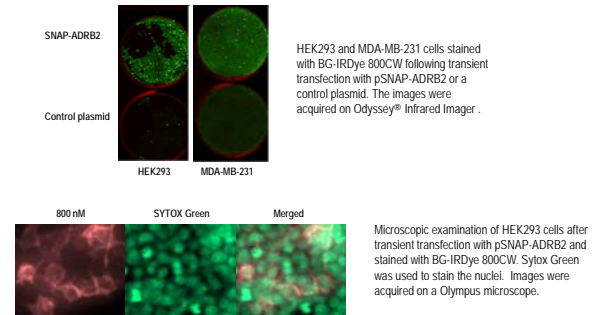


Near-Infrared (NIR) Imaging of a Mouse Tumor Model



A tumor bearing nude mouse was imaged after receiving an intravenous injection of IRDye 800CW-labeled SNAP-EGF. A431 tumor cells (10⁶) were implanted subcutaneously on the right flank of a nude mouse and grown to approximately 0.5 cm in size. Imaging was performed with a Pearl Imager at A) 24 and B) 48 hours post injection of IRDye 800CW-labeled EGF-SNAP. Although higher signal was detected in the tumor at 24 hours, the peak SNR was achieved at 48 hours post injection.

Analysis of SNAP-tag IRDye Substrate by Cell Assays



Conclusions

- In this study, SNAP-tag was successfully used for the generation of an EGF imaging probe by site-specific conjugation with IRDye[®] 800CW for whole cell binding and non-invasive optical imaging of mice.
- We have demonstrated that targeting EGFR is an effective method to study tumor models by *in vivo* near-infrared imaging of small animals.
- Microscopic examination confirmed binding of EGF-SNAP-tag to BxPC3 pancreatic cancer cells overexpressing EGFR. Binding specificity was further verified by competition with unlabeled EGF.
- Biological activity of this EGF-SNAP-tag probe was also determined by stimulation of EGFR phosphorylation.
- This unique system using a self-labeling protein tag and highly sensitive organic dyes provides versatile tools for research and discovery.