HaloTag® Technology: Convenient, Simple and Reliable Labeling from Single Wells to High-Content Screens

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INTRODUCTION

The advent of fluorescent protein labeling technology has revolutionized cell biology by providing the means to study the precise localization and dynamics of proteins within living cells. As this technology has evolved, there have been great advances both in the number of spectrally distinct fluorescent labels available and in the microscopes used to detect them. In fact, elegant studies that include distinct fluorescent entities used together in single cells now have become commonplace in the literature. However, the conventional fluorescent reporting technologies themselves exhibit one fundamental drawback to the scientist: each of the fluorescently labeled proteins requires individual cloning of the gene of interest fused to the fluorescent tag sequence. In other words, researchers must make a separate construct for each "color" of fluorescent tag they wish to put on a protein of interest. The HaloTag® labeling technology eliminates this time-consuming issue.

OVERVIEW OF THE HALOTAG® TECHNOLOGY

Using the HaloTag® Technology, scientists clone their gene of interest into a vector containing the sequence for the HaloTag® protein. When expressed, this protein fusion can be labeled specifically and efficiently with a variety of spectrally distinct fluorescent tags (Table 1). These small tags, called HaloTag® Ligands[a,b], are comprised of a linker that covalently binds to the HaloTag® protein and a fluorescent moiety. Importantly, the HaloTag® protein and these ligands are completely nontoxic to cells. The HaloTag® protein itself has no endogenous eukaryotic equivalent and does not interfere with the proper cellular functioning of fusion partners (1–6). Further, the covalent bond that forms between the HaloTag® protein and the ligand can withstand denaturation, making this system versatile enough to allow reliable fixed-cell and gel-based analyses. The HaloTag® Technology includes a choice of ligands containing affinity tags for solid support (in place of the fluorescent tag), and this expands the use of a single construct to the study of protein:protein and protein:DNA interactions.

RAPID LIVE-CELL LABELING

The HaloTag® labeling technology for imaging offers a quick and simple way to label expressed HaloTag® fusions within live cells. Using this strategy, termed "Rapid" Labeling, the HaloTag® fusion can be labeled with any of a variety of cell-permeant ligands and/or an impermeant one. During the recommended short incubation in the presence of ligand, cell-permeant ligands freely enter cells and their subcellular compartments, covalently attaching to the HaloTag® fusion protein (Figure 1, Panel A). A subsequent wash step allows the unbound ligand to exit cells, resulting in a highly specific signal with very low background noise (Figure 2).

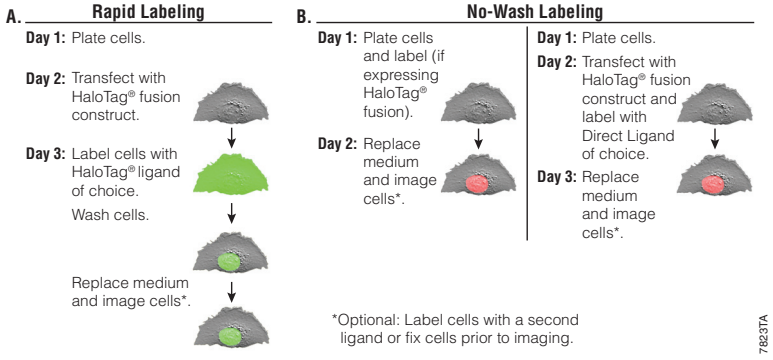

Figure 1. Live-cell labeling with HaloTag® Technology. Schematics show live-cell labeling options. **Panel A.** Rapid labeling. **Panel B.** No-Wash labeling.

Table 1. HaloTag® Ligands for Rapid Labeling.

Labeling Protocols	Product	E_x/E_m (nm)	Size	Cat.#
Rapid Labeling Cell-Permeant	HaloTag® TMR Ligand	555/585	15 µl	G8252
			30 µl	G8251
	HaloTag® Oregon Green® Ligand	496/516 (after hydrolysis)	15 µl	G2802
			30 µl	G2801
	HaloTag® diAcFAM Ligand	494/526 (after hydrolysis)	15 µl	G8273
			30 µl	G8272
	HaloTag® Coumarin Ligand	353/434	15 µl	G8582
			30 µl	G8581
Rapid Labeling Cell-Impermeant	HaloTag® Alexa Fluor® 488 Ligand	494/517	15 µl	G1002
			30 µl	G1001
No-Wash Labeling Cell-Permeant	HaloTag® TMRDirect™ Ligand	555/585	30 µl	G2991
	HaloTag® R110Direct™ Ligand	502/527	30 µl	G3221

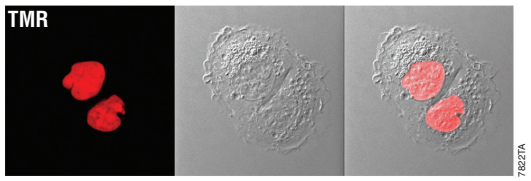

Figure 2. HaloTag® Rapid Labeling results in signal that is robust and specific. Confocal image of U2OS cells expressing HaloTag®-NLS_3 (nuclear localization sequence) and labeled with the HaloTag® TMR Ligand using the Rapid Labeling protocol clearly shows a strong fluorescent signal that is restricted to the nucleus. Panels (left to right) show fluorescence, DIC image and overlay. The image was acquired on a confocal microscope equipped with fluorophore-appropriate filter sets and an environmental chamber in which the cells remained at 37 °C plus CO_2 throughout imaging.

Using the many available fluorescent choices, HaloTag® Rapid Labeling quickly and efficiently labels an expressed protein in a single cell with one or more fluorescent colors. As shown previously (3,4), you can easily pulse the cells with a green fluorescent surface ligand and then chase with a red cell-permeant ligand in order to distinctly label the plasma membrane-bound and internal pools of a single protein. In this way, the HaloTag® Technology provides a simple and powerful means of studying protein translocation and turnover.

NO-WASH LIVE-CELL LABELING

Using the new TMRDirect™ or R110Direct™ Ligands and "No-Wash" Labeling protocol, you can specifically and efficiently label live cells with minimal handling. As shown schematically in Figure 1, the Direct Ligands can be added to the cell medium during the plating or transfection step and left overnight. Cells only need a single medium replacement the following morning prior to imaging.

How is this possible? First, the HaloTag® Direct Ligands are stable; they exhibit a consistent bright signal throughout long-term live-cell imaging experiments or fixation. Second, a longer period of time is allotted for entry of these more dilute ligands into cells. Since the HaloTag® protein is optimized to exhibit a high affinity for the ligands, once inside the cell the Direct Ligands are immediately bound to the HaloTag® protein. Finally, at the concentration used, the Direct Ligands do not require time to wash out; rather this labeling results in a fluorescent signal that is both bright and specific (Figure 3). The HaloTag® Direct Ligands label subcellular structures well, as seen in the robust nuclear signal (Figure 3, Panel A), and exhibit high specificity within cells of numerous types, including neural stem cells (3–5).

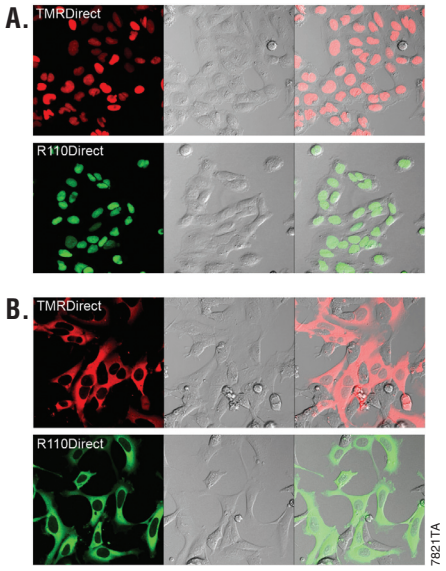

Figure 3. HaloTag® No-Wash Labeling results in signal that is robust and specific. Panel A. Confocal images of U2OS cells expressing HaloTag®-NLS_3 (nuclear localization sequence) and labeled with either the TMRDirect™ or R110Direct™ Ligand using the No-Wash Labeling protocol clearly show strong fluorescent signal that is restricted to the nucleus. **Panel B.** Confocal images of HEK 293 cells expressing HaloTag®-p65 (cytoplasmic in cells at rest) and labeled with the TMRDirect™ or R110Direct™ Ligand using the No-Wash Labeling protocol clearly show strong fluorescent signal that is restricted to the cytoplasm. All panels (left to right) show fluorescence, DIC image and overlay. Images were acquired on a confocal microscope equipped with fluorophore-appropriate filter sets and an environmental chamber in which cells remained at 37 °C plus CO_2 throughout imaging.

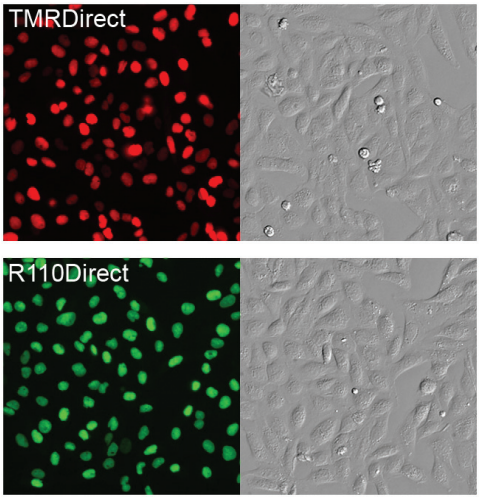

Figure 4. HaloTag® No-Wash Labeling in 384-well format results in signal that is robust and specific. Widefield images of U2OS cells expressing HaloTag®-NLS_3 (nuclear localization sequence) and labeled with either the TMRDirect™ or R110Direct™ Ligand using the No-Wash Labeling protocol clearly show strong fluorescent signal that is restricted to the nucleus. Panels (left to right) show fluorescence and DIC images. Images were acquired on a microscope equipped with fluorophore-appropriate filter sets and an environmental chamber in which cells remained at 37 °C plus CO_2 throughout imaging.

Importantly, the few steps of the HaloTag® No-Wash Labeling protocol coincide with those routinely performed in cell-based experiments; namely plating of cells followed by an overnight incubation and then a replacement of medium prior to experimentation. In addition, cells can be frozen and thawed in the presence of label with no observed deleterious effects (not shown). Thus, the new No-Wash protocol has significant advantages, especially for experiments involving a high number of wells where each additional step requires substantial time, even when automated. HaloTag® Direct Ligands and the No-Wash protocol have been used successfully in a 384-well format. Both live and fixed cells have been imaged by confocal and widefield microscopy in this format. Figure 4 shows live cells imaged by widefield microscopy on a 384-well plate (Greiner Bio-One).

SUMMARY

With the new No-Wash protocol, the HaloTag® Technology now offers all of the convenience of conventional reporters combined with the versatility of the HaloTag® Technology. HaloTag® Technology can thus be applied easily to automated systems involved in high-content analyses, such as those used for drug discovery.

ACKNOWLEDGMENTS

I would like to recognize Dr. Randall Learish for contributing to ligand development and Dr. Georgyi Los for his guidance and support.

REFERENCES

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PROTOCOL

- *HaloTag® Technology: Focus on Imaging Technical Manual* #TM260 Promega Corporation
  www.promega.com/tbs/tm260/tm260.html

ORDERING INFORMATION

Product	Size	Cat.#
HaloTag® TMRDirect™ Ligand	30 µl	G2991
HaloTag® R110Direct™ Ligand	30 µl	G3221
HaloTag® TMR Ligand	15 µl	G8252
	30 µl	G8251
HaloTag® Oregon Green® Ligand	15 µl	G2802
	30 µl	G2801
HaloTag® diAcFAM Ligand	15 µl	G8273
	30 µl	G8272
HaloTag® Coumarin Ligand	15 µl	G8582
	30 µl	G8581
HaloTag® Alexa Fluor® 488 Ligand	15 µl	G1002
	30 µl	G1001

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