

# HaloTag™ technology provides oriented, covalent and specific protein immobilization and labeling *in vivo* and *in vitro*

Marjeta Urh, Dan Simpson, Nidhi Nath, Jacqui Sankbeil, Georgyi V. Los, Chad Zimprich, Natasha Karassina, Randy Learsh, Rachel Freidman-Ohana, Lance P. Encell, Monika Wood, Kate Qin Zhao, Doug Storts, Bob Bulleit, Keith V. Wood\* (Promega Corporation; 2800 Woods Hollow Rd., Madison, WI 53711)  
 Ji Zhu, Mark McDougall, Poncho Meisenheimer, Dieter H. Klaubert (Promega Biosciences, Inc.; 277 Granada Drive, San Luis Obispo, CA 93401)  
 Please address correspondence to Dr. Keith Wood, email address: keith.wood@promega.com

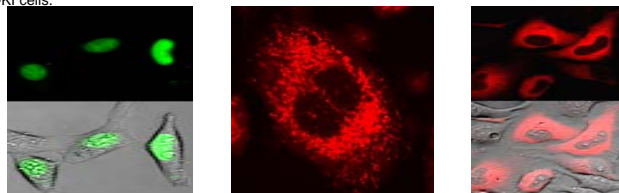


## Abstract

It is becoming clear that surface-based proteomics and protein microarrays will play an important role in the future of proteomics. Successful implementation of surface-based proteomics requires methods that enable stable attachment of proteins while maintaining three dimensional structure and activity. Here we describe a method for specific, covalent and oriented immobilization of proteins onto surfaces. The strategy is based on a fusion protein tag that is a catalytically inactive hydrolase (HaloTag™) designed to form a covalent bond with specific ligands at a rate comparable to the rate of biotin: streptavidin interactions. We chemically modified different surfaces with the HaloTag™ ligand, which allows immobilization of fusion proteins to those surfaces. The rapid and highly specific interaction between the protein and ligand allows immobilization of fusion proteins without the need for prior purification. To demonstrate that protein fusions immobilized via HaloTag maintain their function, we analyzed the activities of several different fusion proteins. Using known protein: protein interactions we show that immobilized proteins interact with their partners with expected specificity. Furthermore the immobilized proteins maintain greater enzymatic activity compared to randomly immobilized proteins. Thus the HaloTag™ technology is well suited for *in vitro* analysis of protein activity. To further our understanding of intracellular processes such as signal transduction pathways, analysis of protein function in living cells is also needed. The method described here can be applied to study protein dynamics and function *in vivo* using ligands coupled to different fluorescent dyes. These ligands can enter the cell and specifically label proteins fused to HaloTag™. Several different protein fusions were made to demonstrate proper subcellular localization and protein migration. These ligands may also be rapidly switched resulting in differential labeling of intracellular proteins (pulse-chase labeling). Through the ability to easily interchange ligands, the HaloTag™ technology eliminates the need to make multiple constructs containing protein of interest fused to different functional tags.

## Imaging HaloTag™ in Living Cells

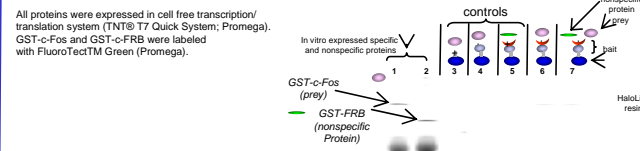
Examples of HaloTag™ localization to different sub-cellular compartments in transiently transfected CHO-K1 cells.



- A) Nucleus  
 B) Mitochondria  
 C) Cytosol
- A) Demonstration of HaloTag™-NLS<sub>3</sub> localization to the nucleus.  
 B) Mito-HaloTag™ localizes to the mitochondrial subunit VIII of human cytochrome C oxidase.  
 C) Demonstration of homogeneous distribution in the cytosol using p65-HaloTag™ fusion.

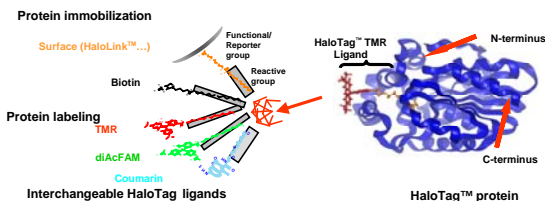
## Detection of Protein Interactions

Pull-down assay using HaloLink™ resin to detect specific interaction between the c-Jun and c-Fos proteins



- Lane 1: In vitro expression of GST-c-Fos (specific prey protein)  
 Lane 2: In vitro expression of GST-FRB (nonspecific protein)  
 Lane 3: resin only + GST-c-Fos  
 Lane 4: resin + HaloTag + GST-c-Fos  
 Lane 5: resin + jun-HaloTag + GST-FRB  
 Lane 6: resin + jun-HaloTag + GST-c-Fos  
 Lane 7: resin + jun-HaloTag + GST-c-Fos + GST-FRB  
 Only the specific interaction between c-Jun-HaloTag and GST-c-Fos were detected, demonstrating specificity of this system.

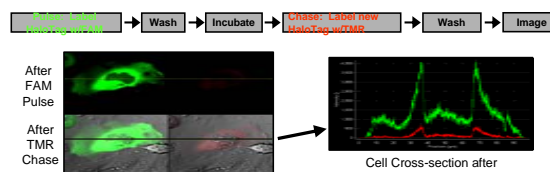
## HaloTag™ Technology



HaloTag™ is a monomeric protein engineered to covalently bind a series of interchangeable ligands. All the ligands have the same reactive group (shown in orange in the protein structure model on the left and highlighted in grey in the ligand structures on the right). Ligands carry different functional/reporter groups which impart different functionalities to the HaloTag™ and its fusion partners. Thus, a single HaloTag™-fusion protein construct can be labeled with multiple colors, immobilized and displayed on a surface, which traditionally requires synthesis of many different clones carrying the protein of interest fused to different tags.

## HaloTag™ technology for Pulse-Chase

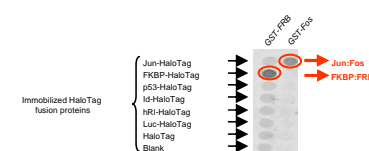
HaloTag technology allows temporal control of labeling.



- At time = 0 the p65-HaloTag fusion protein was labeled with green diAcFAM HaloTag™ ligand.
- Free ligand was washed away and cells were incubated to allow new protein synthesis.
- Newly synthesized protein was labeled with red TMR-HaloTag™ ligand.

## Protein interactions on Glass Slides

Glass slides modified with HaloTag linker can be used to detect protein-protein interactions

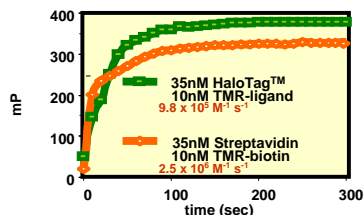


- Seven different bait proteins (HaloTag-fusion) were immobilized onto the chloroalkane modified glass slide.
- All proteins were probed with two different prey proteins.
- Specific protein-protein interactions were detected.

All proteins were expressed in cell free transcription/translation system (TNT® T7 Quick System; Promega). GST-c-Fos and GST-c-FRB were labeled with FluoroTect™ Green (Promega).

## Binding of HaloTag™ Ligand

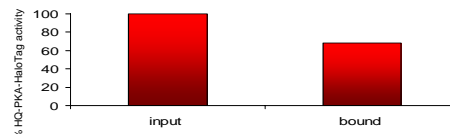
Comparison of binding kinetics of HaloTag™ ligand and streptavidin:biotin using fluorescence polarization.



- Binding of HaloTag™ to its ligand is rapid and irreversible.
- Binding rate is similar to the streptavidin:biotin interaction.

## Enzyme Immobilization on HaloLink™

HQ-PKA-Halo Tag kinase immobilized onto HaloLink maintains its activity.



- Purified HQ-PKA-HaloTag™ kinase was immobilized onto HaloLink™.
- Kinase enzymatic activity was measured and expressed as % of total input activity using Kinase-Glo® (Promega).
- 70 % of the input HQ-PKA-HaloTag™ activity was detected on the HaloLink™-resin.

## Conclusions

- HaloTag™ technology imparts multiple functionalities on a single HaloTag™-protein construct, thus eliminating the need to make multiple clones carrying the protein of interest fused to different tags.
- HaloTag™ enables rapid, specific and covalent labeling and immobilization of HaloTag™-fusion proteins for *in vivo* and *in vitro* applications.
- HaloTag can be used for pulse-chase labeling studies
- HaloTag™ provides high density, stable and oriented immobilization to different surfaces.
- The HaloTag™ technology can be used in a variety of *in vitro* applications including:
  - Protein:protein interaction studies.
  - Detection of enzymatic activity of immobilized proteins.
  - Capture of proteins from complex media.

For Business Development opportunities please contact:  
 Dr. Gary Madsen at 608-298-4820 or gary.madsen@promega.com