Tracking Cellular Protein Localization and Movement in Cells with a Flexible Fluorescent Labeling Technology

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Presentation Overview

- HaloTag® Fusion Technology
  - Design
  - Functionality
  - Ligands
- Imaging Focus on HaloTag® for:
  - Cellular localization studies
  - Protein trafficking
    - Real-time
    - Spatial/Temporal
    - Multiplexing
    - High content screening
What is HaloTag® Technology?
A Unique, Multifunctional Protein Fusion Tag

HaloTag®:
• Engineered 34.1kDa halophilic bacterial hydrolase
• Binds to chloralkane substrate and locks with covalent attachment
• Faster kinetics than the biotin:streptavidin interaction
• No homolog in mammalian cells = no background

HaloTag® Fusion Protein Technology
A Powerful, Multifunctional Fusion Protein Tag

HaloTag® Surfaces/Resins
Capture and Display
- Protein arrays
- Purification
- Interactions

HaloTag® Fluorescent Ligands
Labeling and Detection
- Cellular imaging
- Gel analysis
- Quantitation
- Animal Imaging

HaloTag® Reactive Ligands
Custom Modifications
- Attach to particles, surfaces
- Attach special ligands

Magnetic, non-magnetic resins, glass slides
Many colors of cell permeable & impermeable ligands
e.g. Quantum Dots, PET ligands
HaloTag® Fusion Protein Technology
Many Applications with a Single Fusion Protein

Protein:Protein Interactions

Protein:Nucleic Acid Interactions

Protein Localization & Trafficking

Purify

Protein of Interest

Structural Studies
Enzymatic Assays
Kinetic Studies

Promega Corporation
Multiple Cell Permeable & Impermeable Fluorescent HaloTag® Ligands Available

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Membrane Impermeable</th>
<th>Membrane Permeable</th>
<th>Direct (no wash) Labeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>HaloTag® Coumarin</td>
<td>Y</td>
<td></td>
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<tr>
<td>HaloTag® Alexa Fluor® 488</td>
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<td>HaloTag® Oregon Green®</td>
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<td>HaloTag® diAcFAM</td>
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<td>HaloTag® R110</td>
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<tr>
<td>HaloTag® TMR</td>
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<td>Y</td>
<td></td>
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<tr>
<td>HaloTag® Alexa Fluor® 660</td>
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</tbody>
</table>

- Membrane impermeable ligands label fusion protein where HaloTag® is outside the cells
- Cell permeable ligands label all HaloTag® proteins throughout cells
- Sequential labeling with impermeable then permeable ligands enables differential labeling
Simple Intracellular Labeling and Imaging with HaloTag® Technology

- Transfect HT-ORF expression vector
- Add fluorescent ligand
- Image live or fixed cells
  - Alternate labeling protocol with no-washing
  - Fluorescent Ligand covalently attached to HaloTag® Fusion protein

- ✓ No cytotoxicity
- ✓ Minimal background fluorescence
- ✓ Multiple colors available
- ✓ Cell permeable (complete cell staining)
- ✓ Cell impermeable (cell surface staining)
- ✓ Rapid and easy protocols
HaloTag® Intracellular Protein Localization and Detection Throughout the Cell

|--------------------------|----------------------------------|---------------------------|----------------------|

![Nucleus Image](image1)

![Mitochondria Image](image2)

![Cytosol Image](image3)

![Membrane Image](image4)
HaloTag® Protein Trafficking
Real-time Imaging

Signal
(TNFα, LPS, UV...)

Cell membrane

NFκB Signaling Pathway

Cytoplasm

Nucleus

- HeLa cells expressing p65-HaloTag® labeled with TMR Ligand
- Treated with TNFα
- Imaged (5min/frame; 120min)

HaloTag® Protein Trafficking
Spatial and Temporal Analysis

1. Label with cell **impermeable** ligand
2. Label with cell **permeable** ligand

Trafficking and Assembly of GABA$_A$ Receptor (LGIC superfamily)

In collaboration with Srinivasan Venkatachalan and Cynthia Czajkowski, UW-Madison

γ2L-HaloTag co-expressed with α1 or β2 does not traffic to cell surface

γ2L-HaloTag co-expressed with both α1 and β2 trafficks to cell surface

No Effect on GABA$_A$R Pharmacology
Monitoring GPCR Internalization: HaloTag®-EDG1

The Biology of GPCRs

- G protein activation
- Ligand binding
- GPCR
- β-arrestin
- Trafficking:
  - β-arrestin Translocation
  - Receptor Internalization and desensitization
- Signaling:
  - MAPK, PI3K, AKT
  - Transcriptional control
  - Transactivation (EGFR)

- Second messengers
- Cell responses

- HEK 293 cells expressing pHaloTag®-EDG1 labeled with Alexa488 (surface) and TMR (internal) Ligands
- Treated with S1P
- Imaged (2min/frame; 30min)
Surface Receptor Internalization Assay with HaloTag® pH Sensor

pH Sensor Imaging Based Fluorescence Assay

- HaloTag® pH Sensor: Dark at physiological pH
- HaloTag® pH Sensor: Gain of signal assay
- Live Cell Kinetic
- HaloTag® Dual Functionality: Receptor internalization and recycling

pH Titration and Fluorescence Recovery

- U20S HT-EDG1
- Labeled with HT-pH sensor
- Stimulated with S1P
- Images acquired
Creation of a HaloTag® Ligand for High Resolution (STED) Microscopy

Confirmed ligand specificity for HaloTag® in cells by confocal:

β1 integrin (truncation)-HaloTag® in HeLa Cells

Multiplexing HaloTag® Imaging with Other Methods

- HaloTag® imaging is compatible with:
  - Fluorescent protein fusions
  - Fixing and antibody staining
  - Simultaneous labeling during fixation is also possible

hMGFP–α-tubulin
HaloTag®–NLS$_3$-TMR ligand

p65-HaloTag®-TMR Ligand
Anti-β-tubulin/Alexa 488 2$^{nd}$ Ab
High Content Screening
Whole Cell-based Assays

- Extracellular labeling of ECS-HaloTag® fusion protein with Alexa 488 HaloTag® ligand
- Protein internalization - endosome formation – punctate staining
- Image analysis and quantitation
HaloTag® Imaging Summary
Making Trafficking Studies Possible

✓ Simple, efficient labeling of HaloTag® fusion proteins
✓ No cytotoxicity of HaloTag® fluorescent ligands
✓ Technology enables both temporal and spatial trafficking studies
✓ Intracellular vs. cell surface differential labeling using cell permeable and impermeable labeling ligands
✓ Well-suited to high content screening due to pulse labeling
✓ Multiple label colors available
✓ Same fusion protein used for other applications:
  • Protein:protein interactions
  • Purification – enzyme or structural studies
  • Chromatin IP
Technical Services Scientists are Ready to Help

By phone: 800-356-9526
  • Available 7am-6pm Central M-F

Online Chat@promega.com
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  • Global Chat with Branch office tech serv scientists, too, after hours
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  • Guaranteed answers within 24hr
  • Most responses within 2hrs
Thank You!