



HaloTag® Technology For Protein Expression, Solubilization and Purification

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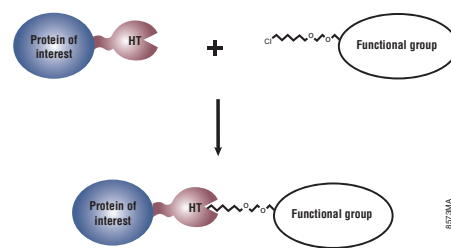
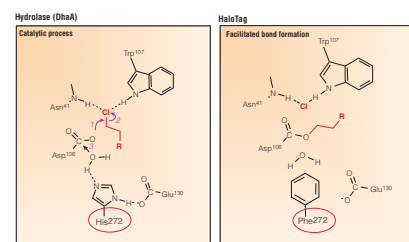
1. Introduction

The HaloTag protein is engineered from a bacterial dehalogenase (MW 34kDa) to covalently attach to a set of chloroalkane ligands with different functional groups, such as fluorescent dyes, biotin and solid surfaces. It has been successfully applied to live cell imaging, protein interactions and protein immobilization.

Designed for structural compatibility with fusion protein partners, HaloTag Technology has the following advantages:

- Enhancement of target protein expression/solubility as compared to GST, MBP and His₆Tag in *E. coli*.
- Covalent immobilization with HaloLink™ Resin for better protein recovery and low non-specific contaminants.
- Optimized TEV linker for better target protein release.
- In-gel detection and quantification of protein expression levels by 1:1 stoichiometric labeling with fluorescent HaloTag ligands.

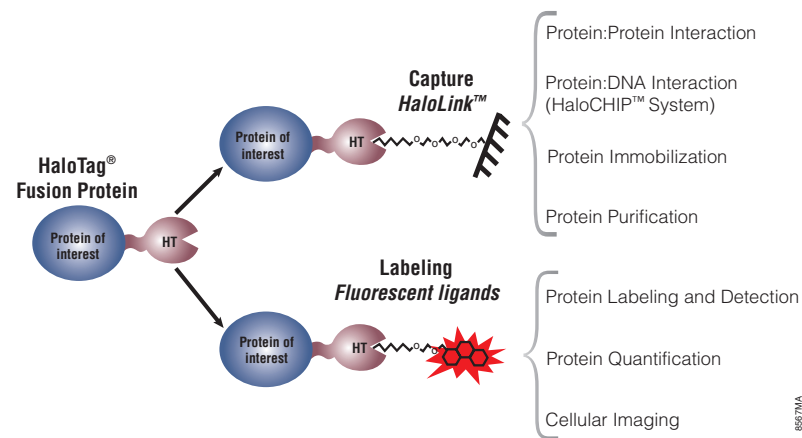
2. HaloTag Technology: A Protein Fusion Tag That Rapidly and Covalently Binds Specific Ligands



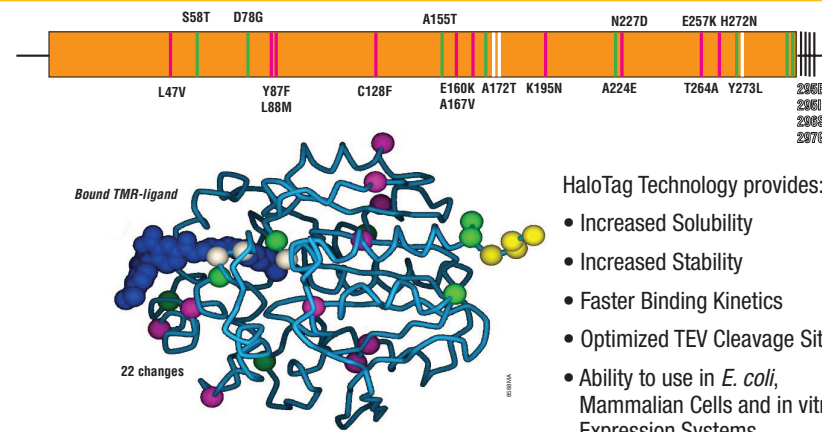
- HaloTag Technology:
- A modified hydrolase from *Rhodococcus rhodocrous*.
 - Forms covalent bond with its designed ligands.

- Functional groups on HaloTag Ligands:
- Fluorescent dyes, e.g., TMR and Alexa Fluor® 488.
 - Solid surfaces: magnetic beads, resin, glass slides.

3. HaloTag Technology—A Versatile Tool for Protein Analysis in vivo and in vitro



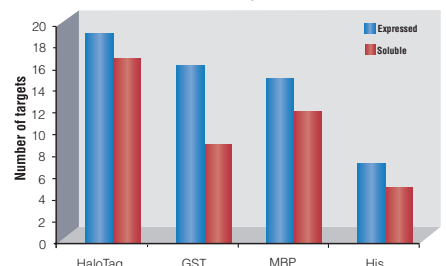
4. Development of HaloTag Technology



- HaloTag Technology provides:
- Increased Solubility
 - Increased Stability
 - Faster Binding Kinetics
 - Optimized TEV Cleavage Site
 - Ability to use in *E. coli*, Mammalian Cells and in vitro Expression Systems

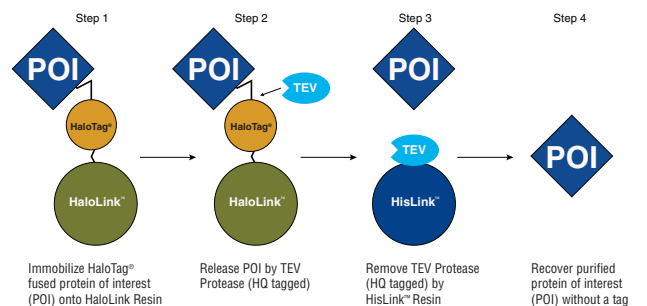
5. HaloTag Technology Enhances Protein Expression/Solubility in *E. coli*

Over 20 difficult-to-express human proteins were tested for expression and solubility in *E. coli* cells (Single Step KRX Competent Cells, Cat.# L3002) with N-terminal HaloTag, GST, MBP, or His₆Tag fusions. HaloTag Technology outperforms GST, MBP and histidine in enhancing protein expression and solubility for this test set. Below is representative data from a subset of these targets.*



Expression and solubility analysis for difficult-to-express human targets. Representative data from a larger set of over 20 difficult-to-express proteins.

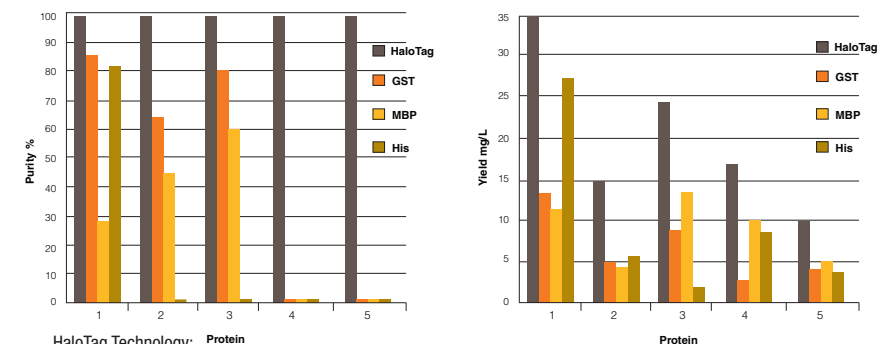
6. Schematics of Protein Purification Using the HaloTag Protein Purification System (Cat.# G6270, G6280)



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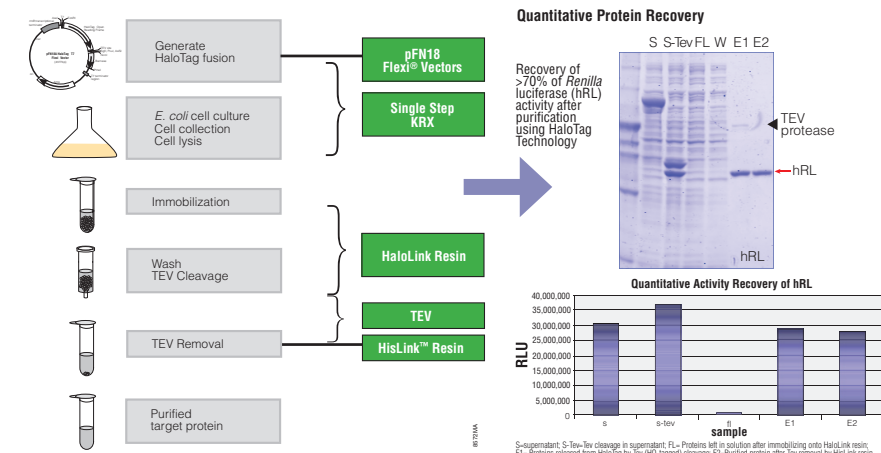
7. HaloTag Technology Purifies Proteins with Higher Yield and Purity from *E. coli*

Higher protein purity and yield with HaloTag Protein Purification System



- HaloTag Technology:
- Enables efficient purification of tag-free, soluble recombinant proteins.
 - Provides superior yield and purity compared to GST, MBP or His₆Tag.

8. Quantitative Protein Recovery from a Streamlined Protein Purification Process Using HaloTag Technology



9. Summary

HaloTag Technology as a tool for protein purification:

- Enhances protein expression and solubility in *E. coli*.
- Enables efficient purification of tag-free, soluble recombinant proteins.
- Produces proteins with superior yield, purity and specific activity.
- Provides a simple method for fusion protein detection and quantification.

Flexi and HaloTag are registered trademarks of Promega Corporation. HaloCHIP, HaloLink and HisLink are trademarks of Promega Corporation. Alexa Fluor is a registered trademark of Molecular Probes, Inc.

* Ohana, R.F., Encell, L.P., Zhao, K., Simpson, D., Slater, M.R., Urh, M., Wood, K.V. (2009) HaloTag7: A genetically engineered tag that enhances bacterial expression of soluble proteins and improves protein purification. *Protein Expr. Purif.* 68(1), 110-120.