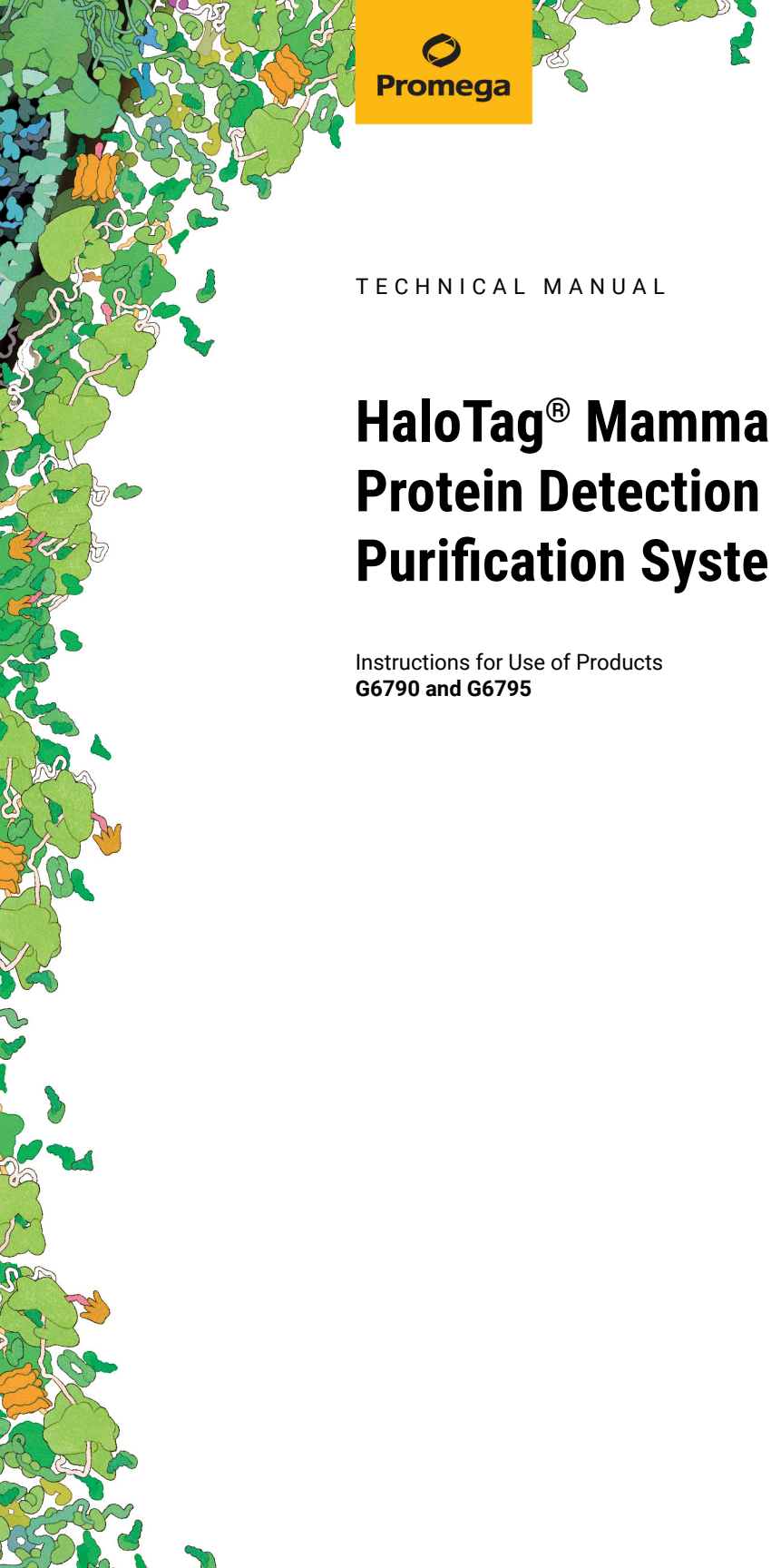

TECHNICAL MANUAL

HaloTag® Mammalian Protein Detection and Purification Systems

Instructions for Use of Products
G6790 and G6795

Revised 7/25
TM348

Promega

HaloTag® Mammalian Protein Detection and Purification Systems

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Manual.
Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The HaloTag® Mammalian Protein Purification System[a] (Cat.# G6790) is based on the HaloTag® technology. HaloTag® is a protein fusion tag engineered to form a highly specific and covalent bond with the HaloLink™ Resin (1–3). The covalent binding coupled with the low nonspecific binding of the HaloLink™ Resin provides superior purity and recovery of recombinant proteins from cultured mammalian cells, even at low expression levels (3). The HaloTag® Mammalian Protein Detection and Purification System (Cat.# G6795) also includes the HaloTag® TMRDirect™ Ligand. The simple-to-use fluorescent detection of HaloTag® fusion proteins facilitates rapid optimization of expression and purification conditions.

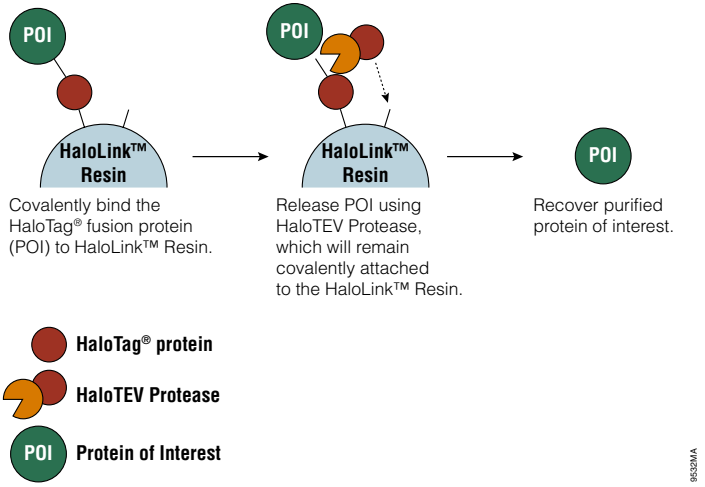

Figure 1. Streamlined purification process leads to higher purity and recovery of recombinant proteins from cultured cells.

2. Product Components and Storage Conditions

PRODUCT	CAT.#
HaloTag® Mammalian Protein Purification System	**G6790**

Each system contains sufficient reagents for 15 purifications from 120ml (or 25 from 40ml) of mammalian cell culture. Includes:

- 2.5ml HaloLink™ Resin (settled resin)
- 200µl HaloTEV Protease
- 2 vials Protease Inhibitor Cocktail, 50X
- 25/pack Spin Columns

PRODUCT	CAT.#
HaloTag® Mammalian Protein Detection and Purification System	**G6795**

Each system contains sufficient reagents for 15 purifications from 120ml (or 25 from 40ml) of mammalian cell culture. Includes:

- 2.5ml HaloLink™ Resin (settled resin)
- 200µl HaloTEV Protease
- 2 vials Protease Inhibitor Cocktail, 50X
- 30µl HaloTag® TMRDirect™ Ligand (0.1mM)
- 25/pack Spin Columns

Storage Conditions: Store the Spin Columns at room temperature. Store the HaloLink™ Resin at +2°C to +10°C. Store the HaloTEV Protease below –65°C and do not subject to more than one freeze/thaw cycle. Dispense into aliquots upon thawing and store aliquots below –65°C. Store Protease Inhibitor Cocktail and HaloTag® TMRDirect™ Ligand at –30°C to –10°C.

3. Preparation of Reagents and Equipment

3.A. Reagents and Equipment to Be Supplied By the User

- (Solution compositions are provided in Section 10.B.)

Protein Expression

- cells for transfection or a stable cell line expressing the desired HaloTag® fusion protein.
- mammalian expression vector encoding the HaloTag® fusion protein in the form of transfection-grade DNA. We recommend using the CMV-driven HaloTag® Flexi® Vectors. See Section 3.C for information on finding compatible Flexi® Vectors.
- equipment and reagents necessary for cell culture (i.e., transfection reagent and growth media).
- Pluronic F-68 polyol (e.g., Fisher Cat.# 2750049) to prevent shearing of cells in spinner flasks.
- Corning® Disposable 125ml Spinner Flasks (e.g., Fisher Cat.# 3152) or T150 Flasks (e.g., Fisher Cat.# 430824).

Cell Lysis

- Dulbecco's Phosphate Buffered Saline (DPBS, e.g., Fisher Cat.# SH30028.02).
- sonicator.
- Detergent Lysis:
  - RQ1 RNase-Free DNase (Cat.# M6101).
  - Mammalian Lysis Buffer (Cat.# G9381); for other compatible detergent lysis buffers see Section 8.
    Do not use RIPA lysis buffer.

Purification

- end-over-end tube rotator and/or a shaking platform.
- HaloTag® Protein Purification Buffer (see composition in Section 3.B).
- Nonidet® P-40 or IGEPAL® CA-630 (Sigma Cat.# 18896), which is chemically indistinguishable from Nonidet® P-40.

3.B. Preparation of Reagents

Protease Inhibitor Cocktail

Reconstitute the Protease Inhibitor Cocktail. Prepare a 50X stock solution of the Protease Inhibitor Cocktail by resuspending in 1ml of 100% ethanol. Store the reconstituted stock at 4°C.

Note: If you are using another protease inhibitor cocktail, do not use one that contains AEBSF because it reduces the binding of the HaloTag® fusion protein to the resin.

HaloTag® Protein Purification Buffer

Choose one of these two buffers:

- 1X PBS (pH 7.5) supplemented with 1mM DTT and 0.005% IGEPAL® CA-630 (Sigma Cat.# 18896).
- 50mM HEPES (pH 7.5) supplemented with 150mM NaCl, 1mM DTT and 0.005% IGEPAL® CA-630 (Sigma Cat.# 18896).

For purification from 40ml, 120ml or 1L cultures, you will need approximately 15ml, 40ml or 80ml of purification buffer, respectively.

Prepare a 10% stock solution of IGEPAL® CA-630 (in water), which will require extensive mixing, and use a final concentration of 0.005% v/v.

Note: DTT and IGEPAL® CA-630 can be omitted, but depending on the protein of interest this may result in reduced protein recovery. See Section 8 for more information on other compatible buffer components.

3.C. Creating HaloTag® Fusion Protein Constructs

Instructions for creating HaloTag® fusion protein constructs can be found in the *Flexi® Vector Systems Technical Manual* #TM254, the *HaloTag® Technology: Focus on Fluorescent Imaging with DMSO-Soluble Ligands Technical Manual* #TM260 and the *HaloTag® Technology: Aqueous-Soluble Ligands Technical Manual* #TM753. We strongly recommend verifying the initial clone by DNA sequencing and examining expression in an appropriate cell line. HaloTag® mammalian vectors are available for creating N-terminus or C-terminus fusion constructs. In these vectors the linker between HaloTag and the protein of interest is optimized to minimize steric hindrance and for efficient cleavage by TEV protease. The linker sequence EPTTEDLYFQSDN contains an optimized Tobacco Etch Virus (TEV) protease recognition site, which allows for proteolytic release of the protein of interest. Because overexpression of any protein may lead to mislocalization within the cell or cell toxicity, we offer a series of HaloTag® Flexi® Vectors with reduced CMV promoter strengths, CMV*d1*, *d2* and *d3*, which enable further optimization of expression levels for individual proteins of interest. Consider using the HaloTag® Flexi® Vectors—CMV Deletion Series Sample Pack (Cat.# G3780) or HaloTag® Cloning Starter System (Cat.# G6050).

Notes:

a. If cloning the HaloTag® protein into another mammalian expression vector, make sure to include the optimized linker as well.

b. Find your protein precloned into a HaloTag® Vector and validated at:
www.promega.com/products/pm/halotag-technology/kazusa-collection/

c. Explore available cloning vectors and kits at:
www.promega.com/products/cloning-and-dna-markers/cloning-vectors-and-kits/

4. Expression and Transfection Protocols

4.A. General Guidelines for Transfection of HEK293/T and CHO Cells

Any method of transfection is compatible with the HaloTag® Mammalian Protein Detection and Purification System. For guidelines on using your transfection reagent of choice, refer to the manufacturer's recommendations. Below are guidelines for using 25kDa linear polyethylenimine (PEI), a cost-effective transfection reagent. PEI (Polysciences, Inc. Cat.# 23966) is a synthetic polycation with transfection efficiencies similar to that of lipopolyamines (4,5). For every new lot of PEI, we recommend optimizing the ratio of DNA:PEI to obtain maximal transfection efficiency. Once this ratio is determined it will apply to any other DNA used, given that the DNA is similar in size. We also recommend optimizing the culture time for each fusion protein. The HaloTag® TMRDirect™ Ligand provides an easy and convenient method for detecting fluorescent proteins and optimizing expression levels; see Section 7.B. for details.

The following protocols are for transfection of HEK293/T cells. Similar protocols can be used for transfecting CHO cells that have been adapted for growth in suspension, see Figure 4. If transfecting CHO cells in spinner flasks, use CHO cells that have been adapted for growth in suspension.

Recommendations for Transfection of HEK-293/T with PEI:

Complete Media: Dulbecco's Modified Eagles Medium-high glucose (DMEM, e.g., Sigma Cat.# D1152) supplemented with 10% Fetalclone I (e.g., Fisher Cat.# SH30080.03) and 1X Penicillin-Streptomycin (e.g., Sigma Cat.# P-4333; optional).

Serum-Free Media (SFM): DMEM.

Pluronic F-68 polyol: Pluronic is a nonionic detergent, which prevents cell shearing and is necessary for cells to thrive in spinner flasks without adaptation.

PEI: Preparation in Section 7.C.

DNA Purity and Concentration: Use transfection-quality DNA (e.g., DNA purified by PureYield™ Plasmid Maxiprep System [Cat.# A2392 or A2393]). The DNA concentration should be approximately 1.0µg/µl.

Final DNA Concentration: 0.8µg of DNA/ml of total culture media.

DNA:PEI Complex Formation: Add the DNA slowly in a dropwise manner to the PEI, and carefully mix via pipetting. The formation of the DNA:PEI complex is done in serum-free media at room temperature.

4.B. Transient Transfection of HEK293/T Cells in Spinner Flasks

1. Start with HEK293/T cells at approximately 70–90% confluency. Seed the spinner flask with 2.5×10^5 cells/ml and the recommended complete media plus 1X pluronic F-68 polyol to prevent cell shearing (see Table 1). The DNA:PEI complexes will comprise 10% of the transfection volume, thus start out with 10% less volume.

Table 1. Transfection of 120ml of Cells in a Spinner Flask at an Optimized DNA: PEI Ratio of 1:3.

HEK293/T Cells	Complete Media + 1X Pluronic F-68 Polyol	DNA/SFM	PEI/SFM
3×10^7 cells	108ml	96µg of DNA 6ml of SFM	288µg of PEI 6ml of SFM

2. Incubate at 37°C, 5% CO_2 with stirring at 60–80rpm for 2–4 hours before adding the DNA:PEI complexes.
3. Prepare the DNA:PEI complexes (see Table 1):
   - Add the DNA at a concentration of 0.8µg/ml of the total transfection volume to SFM (SFM volume is at 5% of the total transfection); see Table 1.
   - In a separate tube, add an optimal amount of PEI (see Section 7.B) to SFM (SFM volume is at 5% of the total transfection); see Table 1.
   - Shake the DNA and PEI solutions separately for 5 minutes at room temperature at 750rpm.
   - Add the DNA/SFM to the PEI/SFM in a dropwise manner, mix gently by pipetting and incubate at room temperature for 20 minutes to ensure proper complex formation.
4. Add the DNA:PEI complexes to the spinner flask containing your cells, and incubate at 37°C, 5% CO_2 with stirring at 60–80rpm. Incubate for the appropriate time, usually 24–72 hours after transfection.
5. For secreted proteins, collect the media and proceed to the purification protocol or freeze at –70°C.
6. For nonsecreted proteins, harvest the cells. Let the cells settle to the bottom, carefully remove the cleared media and collect 25–50ml from the bottom of the spinner. Centrifuge the cells at ~200 × *g* for 5 minutes.
7. Gently wash the pellet 1X with 10ml of DPBS. Centrifuge again at 200 × *g* for 5 minutes. Discard the supernatant, and proceed to cell lysis protocol or freeze the cell pellet at –70°C.

4.C. Transient Transfection of HEK293/T Cells in T150 Flasks

1. Start with HEK293/T cells at approximately 70–90% confluency, and seed the T150 flask with 1 × 10^5 cells/ml and the recommended complete media (see Table 2). We recommend using 40ml total volume for the T150 flask. The DNA:PEI complexes will comprise 10% of the transfection volume, thus start out with 10% less volume.

Table 2. Transfection of 40ml of Cells in a 150cm² Flask at an Optimized DNA:PEI Ratio of 1:3.

HEK293/T Cells	Complete Media	DNA/SFM	PEI/SFM
4 × 10^6 cells	36ml	32µg of DNA 2ml of SFM	96µg of PEI 2ml of SFM

2. Incubate at 37°C, 5% CO_2 overnight before adding the DNA:PEI complexes.
3. Prepare the DNA:PEI complexes (see Table 2):
   - Add the DNA at a concentration of 0.8µg/ml of total transfection volume to SFM (SFM volume is at 5% of the total transfection); see Table 2.
   - In a separate tube, add an optimal amount of PEI (see Section 7.B) to SFM (SFM volume is at 5% of the total transfection); see Table 2.
   - Shake the DNA and PEI solutions separately for 5 minutes at room temperature at 750rpm.
   - Add the DNA/SFM to the PEI/SFM in a dropwise manner, mix gently by pipetting and incubate at room temperature for 20 minutes to ensure proper complex formation.
4. Add the DNA:PEI complexes to the T150 flask containing your cells, and incubate at 37°C, 5% CO_2. Incubate for the appropriate time, usually 24–72 hours after transfection.
5. For secreted proteins, collect the media and proceed to purification or freeze at –70°C.
6. For nonsecreted proteins, harvest the cells. Gently wash the adherent cells with DPBS, then scrape the bottom with a sterile scraper and gently collect the cells.
7. Centrifuge the cells at 200 × *g* for 5 minutes. Discard the supernatant, and proceed to cell lysis protocol or freeze the cell pellet at –70°C.

4.D. General Guidelines for Stable Expression

After generation of a stable cell line expressing the HaloTag® fusion protein, the cells can be expanded in Spinner flasks or T150 flasks. Follow the guidelines for cell seeding (Step 1) and cell harvest (Steps 5–7) described in the protocols for transient expression in Sections 4.B and 4.C.

5. HaloTag® Fusion Protein Purification Protocols

5.A. General Guidelines for Using the HaloTag® Mammalian Protein Purification System

Optimal purification is dependent on several factors including protein expression level, the ratio of cells to HaloLink™ Resin, washing conditions, the efficiency of HaloTEV Protease cleavage and the elution conditions.

Table 3. Guidelines for HaloTag® Protein Purification

Culture	T150 (40ml)	120ml Spinner Flask	1L Culture
HEK293/T cells	2–6 × 10^7 cells	1–3 × 10^8 cells	1–3 × 10^9 cells
Lysis: HaloTag® Protein Purification Buffer or detergent lysis buffer	1ml	5ml	10ml
50X Protease Inhibitor Cocktail	20µl	100µl	200µl
Settled HaloLink™ Resin (25% slurry)	50µl settled (200µl slurry)	150µl settled (600µl slurry)	1.25ml settled (5ml slurry)
HaloTEV Protease (60µl for 1ml settled resin)	3µl	9µl	75µl
Elution volume (2X settled HaloLink™ Resin volume)	100µl	300µl	2.5ml

Notes:

a. The ratio of HaloLink™ Resin and HaloTEV Protease can be scaled up or down proportionally to the culture volume. However, the lysis buffer volume does not need to be scaled proportionally.

b. Batch binding is recommended, since it promotes efficient capture of the HaloTag® fusion protein.

5.B. Preparation of Cell Lysate

This protocol is for purification of proteins expressed in 120ml of media.

Several cell lysis methods have been tested with this system. Provided here are protocols for three lysis methods: sonication, detergent lysis and freeze-thaw lysis.

Cell Lysis by Probe Sonication

This sonication protocol is optimized for the Misonix Sonicator 3000 using a Microtip Probe 419 with output power of 2.5–3.5. Conditions for other sonicators must be determined empirically. Avoid excessive sonication, because this will denature the HaloTag® protein, preventing it from binding to the HaloLink™ Resin.

1. Resuspend cell pellet in 5ml of HaloTag® Protein Purification Buffer (to a concentration of 2–6 × 10^7 cells/ml).
2. Add 100µl of the 50X Protease Inhibitor Cocktail.
3. Sonicate on ice, using 10-second bursts with 10-second cooling time between each cycle for a total of 1 minute at power output of 2.5 (3–6W).

   Note: If using 1L of culture, resuspend the cells in 10ml of purification buffer to 1–3 × 10^8 cells/ml and increase the sonication power output to 3.5 (6–9W).
4. Harvest cell lysate at 10,000 × *g* for 15 minutes at 4°C, and collect the supernatant.

Cell Lysis with Mammalian Lysis Buffer (sold separately)

1. Resuspend cell pellet in 5ml of Mammalian Lysis Buffer (Cat.# G9381; to a concentration of 2–6 × 10^7 cell/ml). For other compatible detergent buffers see Section 8.
2. Add 100µl of the 50X Protease Inhibitor Cocktail and 100µl of RQ1 RNase-Free DNase (Cat.# M6101).
3. Incubate with shaking or rotating at room temperature for 15 minutes.
4. Dilute the lysate 1:3 by adding 15ml of HaloTag® Protein Purification Buffer.
5. Harvest the cell lysate by centrifugation at 10,000 × *g* for 15–30 minutes at 4°C, and collect the supernatant.

Cell Lysis by Freeze-Thaw Treatment

1. Resuspend the cell pellet in 5ml of HaloTag® Protein Purification Buffer (to a concentration of 2–6 × 10^7 cells/ml).
2. Add 100µl of the 50X Protease Inhibitor Cocktail.
3. Lyse the cells with three consecutive freeze-thaw cycles, alternately freezing in a dry-ice/alcohol bath and thawing at room temperature.
4. Add 100µl of RQ1 RNase-Free DNase (Cat.# M6101) and incubate for 15 minutes with shaking or rotating at room temperature.
5. Harvest the cell lysate by centrifugation at 10,000 × *g* for 15–30 minutes at 4°C, and collect the supernatant.

   Note: The cell lysate can be stored at –70°C. Immediately before proceeding to the next step (Section 5.C), thaw the frozen lysate in an ice/water bath.

5.C. HaloTag® Protein Purification

The following protocol is for 120ml of cell culture using 150µl of settled HaloLink™ Resin (600µl of HaloLink™ Resin slurry); see Table 3.

HaloLink™ Resin Equilibration

1. Resuspend the HaloLink™ Resin by thoroughly inverting the bottle.
2. Add 600µl of HaloLink™ Resin slurry to a 15ml conical tube.
3. Centrifuge at 1,500 × *g* for 5 minutes at room temperature, and discard the supernatant.
4. Add 5ml of HaloTag® Purification Buffer to the tube; mix well by inverting, place onto an end-over-end tube rotator/shaker and mix for 5 minutes.
5. Centrifuge as in Step 3, remove supernatant and repeat four more times, for a total of 5 washes. After the last wash, do not remove the supernatant until immediately before use (i.e., HaloTag® Fusion Protein Binding step).

HaloTag® Fusion Protein Binding

6. Discard the supernatant from Step 5, and add cell lysate supernatant (prepared in Section 5.B).

   Note: For secreted proteins add the resin to the collected media.
7. Mix well by inverting, place the tube onto an end-over-end tube rotator/shaker and mix for 1.5 hours at room temperature (22–25°C).

   Note: Make certain that the rotation/shaking speed is sufficient to keep the resin in suspension. If the resin settles it will reduce binding efficiency.
8. Centrifuge at 1,500 × *g* for 5 minutes, and transfer the supernatant into another tube; this is the flowthrough fraction. We recommend keeping this fraction to analyze binding efficiency via HaloTag® TMRDirect™ labeling (see Section 7.A).

   Alternatively, binding can be performed at 4°C for 5 hours or longer (we generally recommend overnight binding).

   If purifying a secreted protein, we recommend overnight binding at 4°C.

Wash

9. Wash the resin with 5ml of HaloTag® Protein Purification Buffer; mix well by inverting, place onto an end-over-end tube rotator/shaker and mix for 10 minutes.

   Note: Make certain that the rotation/shaking speed is sufficient to keep the resin in suspension. If the resin settles it will reduce the washing efficiency.
10. Centrifuge at 1,500 × *g* for 5 minutes. Discard the supernatant.
11. Repeat for a total of 3 washes. After the last wash, do not remove the supernatant until immediately before use in Step 13.

5.C. HaloTag® Protein Purification (continued)

HaloTEV Protease Cleavage/Elution

12. To make the HaloTEV Protease cleavage solution, add 9µl of HaloTEV Protease to 291µl HaloTag® Protein Purification Buffer.

13. Discard the supernatant from Step 11 and add the HaloTEV Protease cleavage solution to the settled resin; mix well. Shake on a mixing rotator or platform shaker for 1.5 hours at room temperature (22–25°C).

    Alternatively, the HaloTEV Protease cleavage/elution step can be performed at 4°C for 5 hours or longer (we generally recommend overnight cleavage).

    Note: Make certain that the shaking speed is sufficient to keep the resin in suspension. If the resin settles it will reduce cleavage efficiency.

14. Collect the supernatant (Elution 1) by centrifuging at 1,500 × *g* for 5 minutes. To ensure resin-free elution, transfer Elution 1 into a Spin Column (inserted into a 1.5ml centrifuge tube). Cap the Spin Column and centrifuge at 10,000 × *g* for 15 seconds.

15. Add an additional 300µl of HaloTag® Protein Purification Buffer to the resin, mix well, and place on a mixing rotator for 30 minutes at room temperature.

16. To collect Elution 2, transfer the resin into the same Spin Column (inserted into a new 1.5ml centrifuge tube). Cap the Spin Column and centrifuge at 10,000 × *g* for 15 seconds.

17. To ensure resin-free elutions, centrifuge both Elution 1 and 2 (10,000 x *g* for 1 minute), and transfer to clean tubes, taking care not to disturb any residual resin at the bottom of the tube. Analyze using SDS-PAGE.

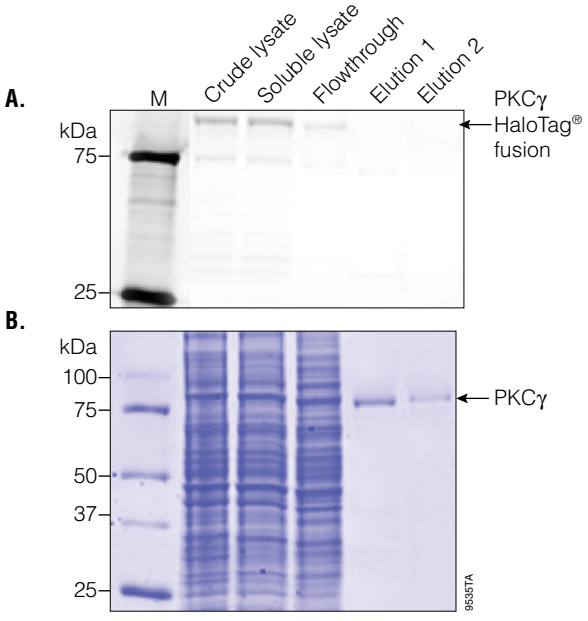

Figure 2. SDS-PAGE analysis of protein purified from 120ml of HEK293T cells. All purification samples including crude lysate, soluble lysate, flowthrough, proteolytic released protein of interest (Elution 1) and the additional wash of the resin (Elution 2) were labeled with HaloTag® TMRDirect™ Ligand prior to gel analysis. **Panel A.** Fluoroimage of the SDS-PAGE gel ($555_{EX}/585_{EM}$). **Panel B.** Coomassie®-stained fluoroimage from Panel A.

6. Guidelines for HaloTag® Fusion Membrane Protein Purification

Membrane proteins have been estimated to comprise 27% of the human proteome (6), while they account for greater than 50% of the known human pharmaceutical targets (7). When purifying membrane proteins, detergents are a vital component. Detergent selection is dependent on solubilization, compatibility with purification technologies, and downstream applications (e.g., crystallization, functional assays). One of the physicochemical characteristics of detergents is the critical micelle concentration (CMC), which is defined as the minimal concentration of detergent to cluster and form micelles. The CMC varies with pH, ionic strength, temperature and with the presence of protein and other detergents (8,9). Detergent compatibility with HaloTag® purification technology is listed in Table 4.

Table 4. Detergent Compatibilty with HaloTag® Purification Technology

Name	Anatrace Cat.#	Molecular Weight	CMC/TBS	3X CMC/ TBS	10X CMC/ TBS	Recommended for Membrane Purification
n-Dodecyl-N,N-Dimethyl-amine-N-Oxide (LDAO)[1]	D360S	229.4	1–2mM (0.023%)	0.069%	0.23%	Yes
n-Nonyl-β-D-Glucopyranoside (NG)[2]	N324S	306.4	6.5mM (0.20%)	0.6%	2.0%	Yes
5-Cyclohexyl-1-Pentyl-β-D-Maltoside (Cymal-5)[2]	C325S	494.5	2.4–5.0mM (0.12%)	0.36%	1.2%	Yes
n-Octyl-β-D-Galactopyranoside (OG)[2]	O311S	292.4	29.5mM (0.86%)	2.58%	8.6%	No
n-Dodecyl-β-D-Maltopyranoside (DDM)[2]	D310S	510.6	0.17mM (0.0087%)	0.0261%	0.087%	Yes
n-Decyl-β-D-Maltopyranoside (DM)[2]	D322S	482.6	1.8mM (0.087%)	0.261%	0.87%	Yes
Cyclofos-7[3]	C518	363.3	0.62mM (0.022%)	0.066%	0.22%	Yes[4]

[1]Zwitterionic. [2]Nonionic. [3]Lipid-like. [4]We do not recommend using Cyclofos-7 in the presence of CHS with HaloTag® purification.

Information on aggregation number, molecular weight of micelle and stability can be found at: **www.anatrace.com**

Cholesteryl Hemisuccinate (Anatrace Cat.# CH210) is compatible with the HaloTag® technology and can be added to the compatible detergents, except for the lipid-like detergent Cyclofos-7. CHS enhances membrane protein stability (10–13); moreover, it usually improves binding of HaloTag® fusion and HaloTEV cleavage efficiency. This usually results in a more overall efficient purification.

6. Guidelines for HaloTag® Fusion Membrane Protein Purification (continued)

We recommend that you make a 10% stock of the detergent or 10% detergent/2% CHS and use this to make the CMC that is necessary for the purification. Use tris-buffered saline (TBS, Appendix 10.B) to make the CMC, 3X CMC and 10X CMC solutions in Table 4.

6.A. Detergent Protocols

To make a 10% detergent stock:

1. Add 3ml of deionized water to a 15ml Corning tube.
2. Add 0.5ml of 2M Tris HCl (pH 8.0) to the water (the final concentration of Tris should be 200mM).
3. Add 0.5 grams of dry detergent to the tube and invert the tube until the detergent goes into solution.
4. Bring solution to 5ml with deionized water.
5. Mix until detergent goes into solution and store at 4°C.

To make 10% detergent/2% CHS stock, we recommend the following protocol (14):

6. Add 3ml of deionized water to a 15ml Corning tube.
7. Add 0.5ml of 2M Tris HCl (pH 8.0) to the water (final concentration of Tris should be 200mM).
8. Add 0.5 grams of dry detergent to the tube and invert the tube until the detergent goes into solution.
9. Add 0.1 gram of CHS to the detergent solution.
10. Sonicate continuously until the solution is hot to the touch and translucent.
11. Bring solution up to 5ml with deionized water.
12. Mix until the solution becomes clear and store at 4°C.

Table 5. Conditions for Each Detergent (+/–) CHS for Binding and Cleavage.

Detergent (+/– CHS)	LADO	NG	Cymal-5	DDM	DM	Cyclofos-7	Fos-Choline-14
Binding Conditions	3X CMC	3X CMC	3X CMC	10X CMC	3X CMC	3X CMC	3X CMC
Cleavage Conditions	CMC	CMC	CMC	3X CMC	CMC	CMC	CMC

6.B. Preparation of Cell Lysate

Cell Lysis by Probe Sonication

Sonication conditions must be determined empirically. Avoid over sonication, because this will denature the HaloTag® protein, preventing it from binding to the HaloLink™ Resin.

1. Resuspend cell pellet from 120ml of culture in 5ml of TBS to a concentration of 2–6 × 10^7 cells/ml.
2. Add 100µl of 50X Protease Inhibitor Cocktail.

   Note: If using another protease inhibitor cocktail avoid those containing AEBSF, which reduces specific capture of HaloTag® fusions.
3. Sonicate on ice, using 10-second bursts with 10-second cooling time in between for a total of 1 minute at approximately 6W of power output.

   Note: If using a 1L culture, resuspend the cells in 10ml of purification buffer to 1–3 × 10^8 cells/ml, and increase the sonication power output to 9W.
4. Pellet the nuclear fraction 500 × *g* for 15 minutes at 4°C and collect the supernatant.
5. Add 2.2ml of 10X CMC detergent, or in the case of DDM use 5ml of 20X CMC, so that binding to the HaloLink™ Resin and solubilization is in the presence of 3X CMC or, if using DDM, is 10X CMC.

6.C. HaloTag® Membrane Protein Purification

HaloLink™ Resin Equilibration

1. Resuspend the HaloLink™ Resin by inverting the bottle.
2. Add 600µl of HaloLink™ Resin slurry to a 15ml conical tube.
3. Centrifuge at 1,500 × *g* for 5 minutes at room temperature and discard the supernatant.
4. Add 1–2ml of 3X CMC detergent to the tube or, in the case of DDM, use 10X CMC. Mix well by inverting, place onto a tube rotator/shaker and mix for 5 minutes.
5. Centrifuge as in Step 3, remove supernatant and repeat two more times, for a total of 5 washes. After the last wash, do not remove the supernatant until immediately before use.

HaloTag® Fusion Binding

6. Discard the supernatant from Step 5 (Section 6.C), and add the cell lysate supernatant (prepared in Section 6.B).
7. Mix well by inverting, place the tube onto a tube rotator/shaker and mix overnight at 4°C.
8. Centrifuge at 1,500 × *g* for 5 minutes and transfer the supernatant into another tube; this is the flow-through fraction. We recommend keeping this fraction to analyze for binding efficiency via HaloTag® TMRDirect™ labeling (see Section 7.A).

6.B. Preparation of Cell Lysate (continued)

Wash

9. Wash the resin with 2–5ml of 3X CMC or, if using DDM, use 10X CMC; mix well by inverting, place onto a tube rotator/shaker and mix for 10 minutes.
10. Centrifuge at 1,500 × *g* for 5 minutes. Discard the supernatant.
11. Repeat for a total of 2 washes.
12. Wash the resin with 2ml of CMC buffer or, if using DDM, use 3X CMC; mix well by inverting, place onto a tube rotator/shaker and mix for 10 minutes.
13. Repeat for a total of 2 washes.
14. After the last wash, do not remove the supernatant until immediately before use.

HaloTEV Protease Cleavage/Elution

Optional: The addition of 1mM DTT usually enhances HaloTEV cleavage.

15. Add 3.6 µl of HaloTEV to 296µl CMC or 3X CMC if using DDM, discard the supernatant from Step 14 and add the cleavage solution to the settled resin; mix well.
16. Shake on a mixing rotator or platform shaker for 4 hours at room temperature or overnight at 4°C.
17. Collect the supernatant (elution) by centrifuging at 1,500 × *g* for 5 minutes, or collect the elution through the spin column (10,000 × *g* for 15 seconds) to eliminate resin contamination.

7. Additional Protocols

7.A. Detection of HaloTag® Fusion Proteins

Fluorescent labeling of HaloTag® fusion protein with the HaloTag® TMRDirect™ Ligand provides a rapid and convenient method to monitor protein expression and follow the purification efficiency. HaloTag® TMRDirect™ Ligand ($555_{EX}/585_{EM}$) is provided with the HaloTag® Mammalian Detection and Purification System (Cat.# G6795) and is also available separately (Cat.# G2991). The covalent bond between the HaloTag® fusion protein and HaloTag® ligand allows for resolution by SDS-PAGE and detection of the fluorescently labeled fusion proteins using a fluorescent scanner. For a complete list of all the available HaloTag® fluorescent ligands, see Section 10.C. For further information regarding HaloTag® labeling refer to the *HaloTag® Technology: Focus on Fluorescent Imaging with DMSO-Soluble Ligands Technical Manual* #TM260 and *HaloTag® Technology: Aqueous-Soluble Ligands Technical Manual* #TM753, available at: **www.promega.com/protocols**

1. Dilute the HaloTag® TMRDirect™ Ligand stock solution (100µM) twofold in DMSO to make a 50µM working solution that can be stored, protected from light, at –20°C; alternatively, the stock solution can be prepared in PBS, but can not be stored.
2. Combine 10µl of lysate containing the HaloTag® fusion protein or the equivalent amount of unbound fraction with 19µl of HaloTag® Protein Purification Buffer and 1µl of 50µM HaloTag® TMRDirect™ Ligand.
3. Incubate at room temperature for 15 minutes protected from light.
4. Add 10µl of 4X SDS gel loading buffer (Section 10.B) and heat at 70°C for 3 minutes.
5. Load 10µl onto an SDS-polyacrylamide gel.
6. Scan the gel on a fluorescence imager using settings appropriate for the HaloTag® TMRDirect™ Ligand ($555_{EX}/585_{EM}$), and quantitate band intensities.

7.B. Optimization of Transfection Conditions

Cell Density: Plate the cells at the recommended density (Table 6), so that the culture reaches 40–70% confluency prior to transfection (overnight incubation).

Final DNA Concentration: 0.8µg of DNA/ml of total culture media.

Ratio of DNA to PEI: The ratio of DNA to PEI will vary depending on the lot of PEI, as well as cell type. We recommend testing ratios from 1:1.5 to 1:3.5 (DNA:PEI) for optimal expression.

DNA:PEI Complex Formation: Add the DNA slowly in a dropwise manner to the PEI, carefully mixing via pipetting. The complexes are formed in serum-free media at room temperature. The DNA:PEI complexes should always comprise 10% of the total volume.

7.B. Optimization of Transfection Conditions (continued)

Table 6. Plating Recommendations.

Plate Type	96-Well	48-Well	24-Well	12-Well	6-Well
Surface Area (cm^2)	0.35	1.0	1.9	3.8	9.6
Complete Media with Cells (µl)	81	234	450	900	2250
Serum-Free Media with DNA and PEI (µl)	9	26	50	100	250
Approximate Cell Density (cells/well)	9.2×10^3	2.6×10^4	0.5×10^5	1.0×10^5	2.5×10^5

Protocol for Optimization of Small-Scale Transfection Using a 24-well Plate

1. Culture the cells in the appropriate media. Optimal protein expression will be determined by adding the HaloTag® fluorescent ligand directly to the media at a final concentration of 100nM. Transfection efficiency can be estimated using a fluorescent microscope and oscillating between the bright field and fluorescent field. The HaloTag® TMRDirect™ Ligand ($555_{EX}/585_{EM}$) is provided with the system (Cat.# G6795) and is also available separately (Cat# G2991). Alternatively, you can use HaloTag® R110Direct™ Ligand ($502_{EX}/527_{EM}$; Cat# G3221) or, for a complete list of all available HaloTag® fluorescent ligands, see Section 10.C. For more details on cell imaging refer to the *HaloTag® Technology: Focus on Fluorescent Imaging with DMSO-Soluble Ligands Technical Manual* #TM260 and *HaloTag® Technology: Aqueous-Soluble Ligands Technical Manual* #TM753, available at: **www.promega.com/protocols**
2. Plate cells on two 24-well plates at the recommended density (see Table 6), approximately 24 hours prior to transfection. One plate is for determining protein expression per well, and the other plate is for determining cell viability.

   Note: Determining cell viability is optional.
3. Immediately before transfection, prepare the DNA:PEI complexes by incubating 0.4µg of DNA/well in 25µl of serum-free media.

   Table 7. Ratio of DNA:PEI. (Recommendation for a 24-well plate using 0.4µg of DNA/well).

Ratio of DNA:PEI	1:1.5#	1:2.0	1:2.5	1:3.0	1:3.5
PEI/Well	0.6µg	0.8µg	1.0µg	1.2µg	1.6µg
Serum-Free Media (SFM)/Well	25µl	25µl	25µl	25µl	25µl

4. Mix the separate DNA/SFM and PEI/SFM solutions for 5 minutes at room temperature at approximately 750rpm. Then add the DNA solution to the PEI solution and mix slowly by pipetting.
5. Let the complex formation proceed for 20 minutes at room temperature.

6. Add 50µl of the DNA:PEI complex to each well.
7. Harvest the cells, taking time points between 24 and 72 hours.

Determining Protein Expression Levels and Cell Viability

Often a significant reduction in cell viability correlates with a slight decrease in protein expression (transfection efficiency). We recommend choosing a DNA:PEI ratio that provides optimal expression and minimal reduction in cell viability. Use one 24-well plate to determine protein expression level and the other to determine cell viability.

Determining Protein Expression Level

1. Slowly remove the media, and carefully wash the cells with DPBS.
2. After removing the DPBS, add 100µl of the prepared lysis buffer to each well. Lysis buffer: 0.1X Mammalian Lysis Buffer (Cat.# G9381) + 1:50 dilution of RQ1 RNase-Free DNase (Cat.# M6101). If the HaloTag® fluorescent ligand was not added to the media when the cells were plated, add it to the lysis buffer at a final concentration of 100nM.
3. Slowly shake the plate at room temperature for 60 minutes.
4. Add 32.5µl of 4X SDS gel loading buffer, mix and collect.
5. Heat samples at 70°C for 3 minutes; analyze 10µl of the samples on an SDS-PAGE gel, and scan the gel on a fluorescent scanner using the appropriate setting (HaloTag® TMRDirect™ Ligand, $555_{EX}/585_{EM}$ or HaloTag® R110Direct™ Ligand, $502_{EX}/527_{EM}$).
6. Quantitate the bands, and determine the optimized ratio of DNA:PEI. Once the optimal ratio for the lot of PEI has been determined, it usually will be optimal for any transfection-grade DNA.

Determining Cell Viability (optional):

The second plate is used to determine cell viability, for example using CellTiter-Fluor™ Cell Viability Assay (Cat.# G6080). The CellTiter-Fluor™ Cell Viability Assay can be multiplexed with the HaloTag® TMRDirect™ Ligand and the HaloTag® R110Direct™ Ligand. For a detailed protocol, refer to the *CellTiter-Fluor™ Cell Viability Assay Technical Bulletin*, #TB371 available at: **www.promega.com/protocols**

1. For a 24-well plate, add 0.4ml of the CellTiter-Fluor™ Cell Viability Assay substrate/buffer to each well, mix briefly and incubate at 37°C for 1 hour.
2. Transfer 100µl from each well (in triplicate) to a white, clear bottom 96-well plate and read in a fluorescent plate reader ($400_{EX}/505_{EM}$).

7.B. Optimization of Transfection Conditions (continued)

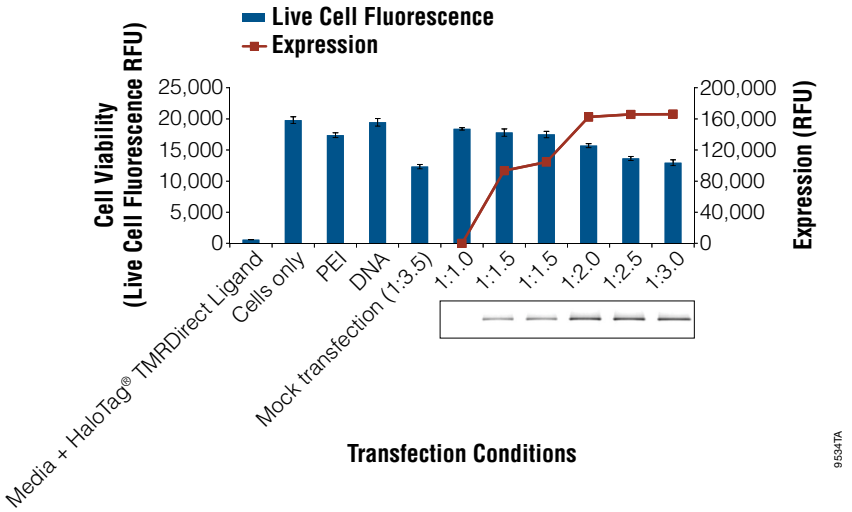

Figure 3. Expression level of PI3KγKinase versus cell viability for HEK293T Cells. Expression levels are quantitated from the fluorescently scanned gel. In this example either the ratio of 1:2.5 or 1:3 is acceptable for large-scale transfections as it maximizes expression with a minimal reduction in cell viability.

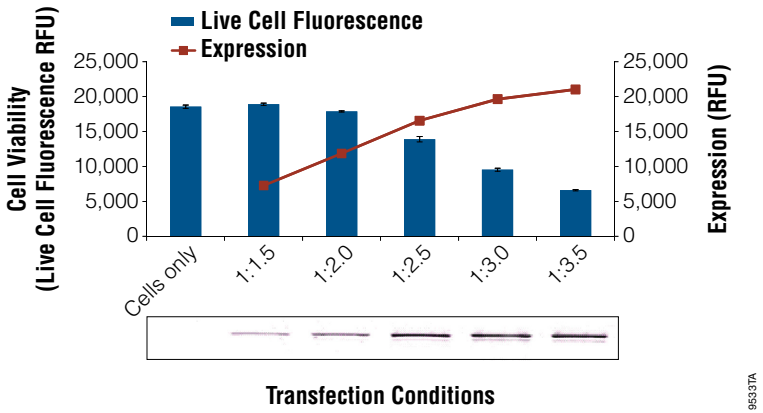

Figure 4. Expression level of PI3KγKinase versus cell viability for CHO Cells. The expression levels are quantitated from the fluorescently scanned gel. In this example either the ratio of 1:2.5 or 1:3 is acceptable for large-scale transfections as it maximizes the expression with a minimal reduction in cell viability.

7.C. Preparation of 1mg/ml PEI (Polyethylenimine)

1. Add 50mg of PEI to a beaker, adding approximately 40ml of deionized water.
2. While stirring, add 6N HCl dropwise to the solution until it is ~ pH 2.0.
3. Stir the PEI solution until it is dissolved, maintaining the pH at ~ 2.0 with HCl.
4. Add 6N NaOH dropwise until the solution reaches pH 7.0.
5. Adjust the final volume to 50ml with deionized water.
6. Pass the solution through a 0.22 micron filter.
7. Aliquot to desired volumes using aspectic technique.
8. Store the aliquots at –20°C or –70°C.

8. Reagent Compatibility Table

Reagent	Acceptable Concentration	Effects
DTT	1mM can be included in HaloTag® Protein Purification Buffer in all steps.	Reduces background caused by intermolecular disulfide linkages and can enhance HaloTEV Protease cleavage.
EDTA	0.5–1mM can be included in HaloTag® Protein Purification Buffer in all steps.	Assists in TEV protease cleavage; inhibits nuclease and metalloprotease.
IGEPAL® CA-630	0.005% can be included in HaloTag® Protein Purification Buffer in all steps.	Prevents the HaloLink™ Resin from sticking to plasticware, reduces nonspecific binding and can lead to higher protein recovery.
Protease Inhibitor Cocktail	The Protease Inhibitor Cocktail provided with the kit is recommended to prevent proteolysis.	If using another protease inhibitor cocktail, avoid ones containing AEBSF, which reduces specific capture of HaloTag® fusion protein by the HaloLink™ Resin.
Glycerol	Up to 5% during binding, but avoid glycerol in the washing and elution steps.	Glycerol reduces the diffusion rate and may lead to low recovery.
Urea/guanidine	Do not use.	Denaturants will interfere with HaloTag® fusion protein binding, which requires native, folded proteins.
NaCl	50–150mM NaCl is recommended throughout the purification.	Higher concentrations (up to 500mM): • May reduce specific capture of HaloTag® fusion protein by the HaloLink™ Resin. • Can reduce HaloTEV Protease cleavage efficiency. • Can be used for protein elution after HaloTEV Protease cleavage, during the Elution 2.

8. Reagent Compatibility Table (continued)

pH	Notes	Effects
pH 6.5–8.0	Can be used throughout the purification.	Compatible with HaloTag® fusion protein binding and HaloTEV Protease cleavage.
pH 4	Do not use.	HaloTag® fusion protein binding is inhibited below pH 5.
pH 10	Do not use.	Not compatible with the HaloLink™ Resin.
Non-ionic Detergents Reduce Nonspecific Binding Due to Hydrophobic Interactions		
CHAPS	0.01%	Higher concentrations can reduce diffusion rate and may lead to low recovery. Detergents also may reduce TEV protoeolytic cleavage. The detergent effect may be protein-dependent and needs to be determined empirically.
Brij35	0.05%	
Triton® X-100	0.05%	
NP-40 (IGEPAL® CA-630)	0.05%	
Metals		
Mg^{2+}	20mM	Compatible with HaloTag® fusion protein purification.
Ca^{2+}	20mM	Compatible with HaloTag® fusion protein purification.
Zn^{2+}	Not recommended	Inhibits TEV cleavage.
Detergent-Based Lysis for Mammalian Cells		
Mammalian Lysis Buffer (Cat.# G9381)	Compatible for cell lysis and binding.	Dilute the lysate threefold with HaloTag® Protein Purification Buffer to enhance the binding to the HaloLink™ Resin. Wash extensively after protein immobilization to remove any factors that may interfere with HaloTEV Protease cleavage.
CelLytic™ Buffer (Sigma)		
M-PER lysis buffer (Thermo)		
RIPA buffer	Do not use.	Reduces binding of HaloTag® fusion protein onto the HaloLink™ Resin, inhibits HaloTEV Protease and reduces protein recovery.

9. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptoms	Causes and Comments
No/low protein expression	Label the lysate with a HaloTag® fluorescent ligand to determine expression levels.
	Optimize transfection, culture and growth conditions (i.e., ratio of DNA:transfection reagent, medium composition and expression time).
	Protein is toxic to the cell. Reduce expression level by reducing expression time or using the Flexi® Vectors with truncated CMV promoter.
Protein appears insoluble	The cell lysis is not complete or the lysis conditions are not optimized.
Protein does not bind to HaloLink™ Resin or binding is inefficient	HaloLink™ Resin mishandled or used improperly: • HaloLink™ Resin is not equilibrated properly. Equilibrate the resin thoroughly with HaloTag® Protein Purification Buffer.
	Cell lysis conditions are inefficient: • Change lysis conditions. • Lysate is too viscous. Treat the lysate with DNases. Do not add EDTA during treatment with DNase. • Samples were sonicated too long or overheated during sonication. Decrease sonication time and output settings. Keep all samples on ice during sonication.
	Improper binding conditions: • Expression level is high: thus the resin is saturated. Increase amount of resin. • Binding time is not sufficient. Increase binding time, e.g., overnight at 4°C. Binding conditions not optimal. Optimal pH range is pH 6.5–8.0. Check pH of the lysate if necessary. • Some additives used in the cell lysate may reduce the specific binding of the HaloTag® fusion protein to HaloLink™ Resin. See Reagent Compatibility Table for details. • Target protein forms a multimer. Dilute the cell lysate prior to binding. • The structure of the target protein might interfere with the reactive site of the HaloTag® fusion protein. Alter the directionality of the fusion.

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9. Troubleshooting (continued)

Symptoms	Causes and Comments
Poor protein elution after HaloTEV Protease cleavage.	Insufficient HaloLink™ Resin equilibration: • The HaloLink™ Resin should be washed 5X prior to use. HaloTEV Protease cleavage is not efficient: • Check the TEV cleavage site. The P1´ position cannot be a proline, and other residues such as T, W, R, L, E, I might be cleaved less efficiently by TEV protease (15). Use the cleavage site in the pFN14A/K and pFN21A/K HaloTag® Flexi® Vectors, which have an optimized TEV recognition site. • Increase the reaction time of HaloTEV Protease cleavage, e.g., overnight at 4°C. • Increase the amount of HaloTEV Protease in the cleavage reaction. • TEV protease is a cysteine protease. Its activity can be enhanced by DTT and inhibited by divalent metals (e.g., Zn^{2+} and Cu^{2+}). • Additives used in the HaloTEV Protease cleavage reaction might inhibit the activity of HaloTEV Protease; avoid these additives if possible (see Section 8, Reagent Compatibility Table). • Detergents might inhibit the TEV protease (16,17) and the effect may be target-dependent. Omit detergent in cleavage reaction or replace with a compatible detergent. Protein is retained by the HaloLink™ Resin: • HaloTag® protein is negatively charged at pH 7.5. Positively charged proteins at this pH can interact with the HaloTag® protein through ionic interaction. Increase the ionic strength of the elution buffer (E2) by adding NaCl (i.e., up to 500mM final concentration). • Very hydrophobic proteins may interact nonspecifically with HaloLink™ Resin. They also may precipitate under high salt conditions. Using a nonionic detergent (i.e., IGEPAL® CA-630; 0.005–0.05%) during elution may enhance protein recovery. • Add HaloTag® Protein Purification Buffer to the resin, and incubate at 4°C overnight to release the protein from the resin.

Symptoms	Causes and Comments
Multiple bands or contaminant protein co-elute with target of interest	Elution 1 and/or Elution 2 contain resin contamination. Centrifuge at 10,000 × *g* for 1 minute and transfer to new tubes.
	Co-elution of HaloTEV Protease (81.5kDa) caused by saturated resin. To remove the contaminating HaloTEV Protease, add equilibrated HaloLink™ Resin to Elution 1 and bind for 30 minutes with constant mixing.
	Protein is degraded. Add Protease Inhibitor Cocktail (Cat.# G6521) to the wash buffer and, if necessary, to the final eluted sample. Perform all steps at 4°C throughout the protein purification process.
	Check for premature stops in the sequence. Eliminate them if possible.
	Additives used in the binding process, such as glycerol, high salt and certain detergents at high concentrations, can promote nonspecific adsorption/aggregation of proteins onto the HaloLink™ Resin. Do not use these additives if possible (see Reagent Compatibility Table).
	Add freshly prepared 0.005% IGEPAL® CA-630 to the HaloTag® Protein Purification Buffer to reduce nonspecific binding to the resin; IGEPAL® may be unstable.
	Too much HaloLink™ Resin used for capture. Reduce the amount of HaloLink™ Resin used.
	Increase wash stringency: • Increase wash volume. • Increase the wash stringency by raising the salt concentration or adding detergents to the wash buffer. Do not use high salt and high detergent at the same time. • Wash with different conditions, such as high-salt followed by low-salt solutions.
	Excessive sonication can lead to nonspecific protein binding to the resin. Use optimized sonication conditions or other mild cell breakage methods.
	Add reducing agent such as DTT to reduce disulfide bond-linked contaminants.
	Protein cofactors that are required for protein function or proper folding might copurify.

9. Troubleshooting (continued)

Symptoms	Causes and Comments
Multiple bands or contaminant co-elute with target of interest (continued)	Co-elution of tubulin (50kDa). Prior to the proteolytic cleavage wash the resin for 30 minutes with a low-salt buffer (≤50mM NaCl).
Protein precipitated	Excessive sonication or improper cell lysis conditions may lead to denaturation or aggregation of target protein. Use proper cell lysis conditions for optimal protein recovery.
	The pI of target protein is close to 7.5; use a different pH for HaloTEV Protease cleavage and elution (pH 6.5–8.0). See Section 8, Reagent Compatibility Table.
	High salt will precipitate hydrophobic protein. Reduce the concentration of salt or include detergent in elution. Other proteins might need high salt to stay in solution. Determine these conditions empirically for each target of interest if necessary.
	Include a reducing agent such as DTT to reduce aggregation formed by intermolecular disulfide bonds.

10. Appendix

10.A. References

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10.B. Composition of Buffers and Solutions

Supplied with System

HaloLink™ Resin
25% slurry in 25% ethanol

Protease Inhibitor Cocktail
Reconstituted 50X stock will contain:

5mM	benzamidine HCl
2.75mM	phenanthroline
0.5mM	bestatin
1mM	leupeptin
0.25mM	pepstatin A
50mM	PMSF

To Be Supplied By the User

4X SDS-PAGE gel loading buffer

240mM	Tris-HCl (pH 6.8)
3mM	bromophenol blue
50%	glycerol
400mM	dithiothreitol
2%	SDS

1X PBS buffer (pH 7.5)

137mM	NaCl
2.68mM	KCl
1.47mM	KH_2PO_4
8.1mM	Na_2HPO_4

Tris-buffered saline (TBS)

50mM	Tris-HCl (pH 7.5)
150mM	NaCl

Optional: (Can enhance HaloTEV protease cleavage).

1mM	DTT

To prepare, dissolve 6.05g Tris and 8.76g NaCl in 800ml of H_2O. Adjust pH to 7.5 with 1M HCl and make volume up to 1L with H_2O.

TBS is stable at 4°C for 3 months.

10.C. Related Products

HaloTag® Flexi® Vectors for Protein Expression in Mammalian Systems

The HaloTag® expression vector collection can be found at:
www.promega.com/products/vectors/protein-expression-vectors/

Cloning Vectors

The list of available cloning vectors and kits can be found at:
www.promega.com/products/cloning-and-dna-markers/cloning-vectors-and-kits/

HaloTag® Ligands for Imaging

Learn about the fluorescent HaloTag® ligands that can be used for imaging at:
www.promega.com/products/protein-detection/protein-labeling/janelia-fluor-halotag-ligands/

Reagents for Protein Expression and Purification from Mammalian Cells

Find protein purification kits at: **www.promega.com/products/protein-purification/protein-purification-kits/**

See the available protein expression kits at: **www.promega.com/products/protein-expression/**

11. Summary of Changes

The following changes were made to the 7/25 revision of this document:

1. Removed discontinued Cat.# G6799.
2. Updated cover page and replaced document font.
3. Removed Table 1 (Section 3.C) and renumbered the remaining tables.
4. Edited Section 10.A.
5. Made minor text edits.