TECHNICAL MANUAL

Magne® HaloTag® Beads

Instructions for Use of Products
G7281 and G7282

Magne® HaloTag® Beads are not recommended for conjugation to the Protein G HaloTag® Fusion Protein (Cat.# G7291). We recommend the Magne® Protein G Beads for antibody purification applications.

Revised 12/15
TM291

Magne® HaloTag® Beads

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

Promega

1. Description

The Magne® HaloTag® Beads[a,b] provide a convenient method to covalently capture HaloTag® fusion proteins with magnetic particles for protein pull-downs and purification. These magnetic beads offer a high binding capacity (≥20mg/ml) for purified HaloTag® fusion proteins with low nonspecific protein binding. Characteristics of the Magne® HaloTag® Beads are listed in Table 1. The magnetic properties allow streamlined protein purification, eliminates the need for multiple centrifugation steps and facilitates automated applications on robotic platforms.

Table 1. Magne® HaloTag® Beads Characteristics.

Composition	Magnetic particles encapsulated with macroporous cellulose
Particle Size	50–80µm
Binding Capacity	≥20mg of purified HaloTag® fusion protein per milliliter of settled particles
Formulation	20% slurry in 20% ethanol

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Magne® HaloTag® Beads	**1ml**	**G7281**
	5 × 1ml	**G7282**

Storage Conditions: Store the Magne® HaloTag® Beads at 2–10°C. Do not freeze. Do not allow beads to dry during storage or use.

3. Before You Begin

The HaloTag® technology can be used to purify and label a protein of interest that is fused to the HaloTag® protein (34kDa). Expression-ready HaloTag® Flexi® Vectors based on the pFN21A HaloTag® CMV Flexi® Vector (Cat.# G2821) can be purchased from the Kazusa Collection (see **www.promega.com/FindMyGene/**).

Fusion proteins may be expressed in cell-based and cell-free systems, depending on the downstream application. The chosen protein expression system will dictate the regulatory elements that must be present in the expression vector for optimal expression. More information about choosing the appropriate expression vector can be found at: **www.promega.com/resources/pubhub/promega-notes-2008/expression-of-fusion-proteins-how-to-get-started-with-the-halotag-technology/**

1. Use of the Magne® HaloTag® Beads requires a magnetic stand. These beads can be used with a variety of magnetic stands (see Section 7.D) to process 1 to 96 samples.
2. Be sure that the Magne® HaloTag® Beads remain in suspension during binding and wash steps for optimal protein yield and purity. The shaking speed must be sufficient to maintain the beads in suspension. If necessary, pipet the beads to maintain them in suspension. If the beads settle or dry, binding and washing efficiency will be reduced.

4. HaloTag® Protein Purification Protocols

HaloTag® purification and pull-down technologies are based on the HaloTag® protein (34kDa), a fusion tag that is engineered to form a specific and covalent bond with the Magne® HaloTag® Beads. The covalent binding coupled with the low nonspecific binding of the Magne® HaloTag® Beads provides superior purity and recovery of recombinant proteins even at low expression levels. Covalent binding also minimizes protein loss during wash steps. The HaloTag® protein also may enhance expression and solubility of recombinant proteins in *E. coli* (1).

During purification, the protein of interest can be released from the beads by HaloTEV Protease (Cat.# G6601) cleavage at the optimized TEV recognition site. Proteolytic release yields a HaloTag®-free protein of interest, while the HaloTag® protein and HaloTEV Protease remain covalently attached to the Magne® HaloTag® Beads (Figure 1).

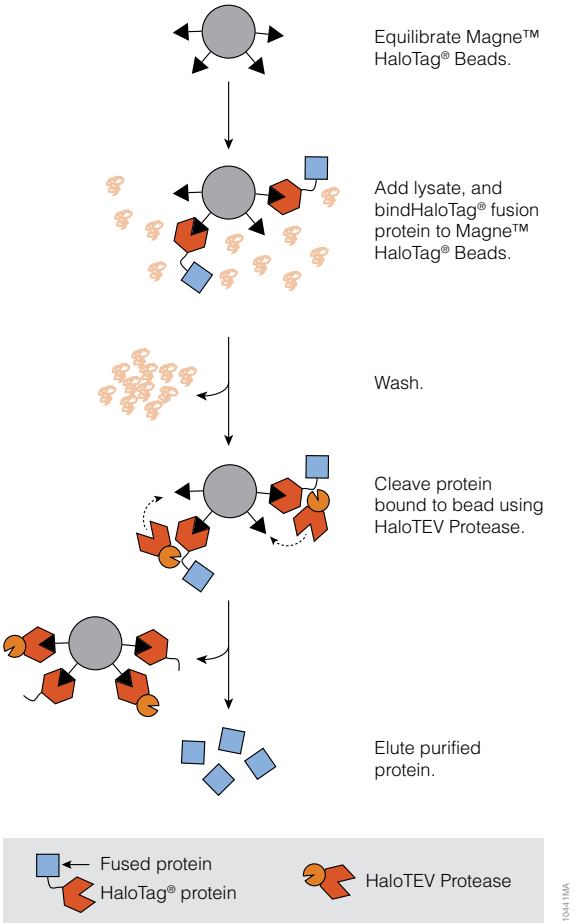

Figure 1. Schematic diagram of the HaloTag® fusion protein purification protocol using Magne® HaloTag® Beads.

4. HaloTag® Protein Purification Protocols (continued)

Purification conditions may require optimization. Optimal purification conditions depend on several factors including protein expression level, the ratio of cells to Magne® HaloTag® Beads, washing conditions, efficiency of HaloTEV Protease cleavage and elution conditions.

The pH range for HaloTag® fusion protein binding is pH 6.5–8.0. The recommended NaCl concentration range is 50–150mM.

In these protocols, all steps are performed at room temperature (22–25°C) unless otherwise noted. Binding and protease cleavage may be performed at 4°C; however, the binding or cleavage time may need to be optimized.

If you are using a protease inhibitor cocktail other than the 50X Protease Inhibitor Cocktail (Cat.# G6521), do not use one that contains 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) because AEBSF reduces the binding of the HaloTag® fusion protein to the beads. For information about other reagents that interfere with binding, see Section 7.A.

For more information and for other methods to lyse cells, refer to the *HaloTag® Mammalian Protein Detection and Purification Systems Technical Manual* #TM348.

For more information about HaloTag® fusion protein purification from *E. coli*, refer to the *HaloTag® Protein Purification System Technical Manual* #TM312.

4.A. Automated and Manual Purification of HaloTag® Fusion Proteins from *E. coli* in a 96-Well Plate

Optional: Treatment with 2mM ATP and 10mM magnesium sulfate ($MgSO_4$) or magnesium acetate [$Mg(CH_3COO)_2$] can reduce copurification of *E. coli* chaperonins (e.g., DnaK [70kDa], DnaJ [37kDa], GrpE [40kDa], GroEL [57kDa] and GroES [10kDa] (2). Incubate the cell lysate in high-throughput *E. coli* purification buffer with 2mM ATP and 10mM $MgSO_4$ or 10mM $Mg(CH_3COO)_2$ for 10 minutes at 37°C prior to protein purification, or incubate the Magne® HaloTag® Beads in high-throughput *E. coli* purification buffer with 2mM ATP and 10mM $MgSO_4$ or $Mg(CH_3COO)_2$ for 30 minutes, as described in Step 14.c, followed by a high-throughput *E. coli* purification buffer wash, as described in Step 14.d.

During mixing steps, the shaking speed must be sufficient to maintain the beads in suspension. If the beads are allowed to settle or dry, binding, washing and proteolytic cleavage efficiencies will be reduced.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 7.C.)

- Single Step KRX cells expressing the HaloTag® fusion protein of interest
- LB medium with the appropriate antibiotic
- late auto-induction medium
- *E. coli* lysis buffer
- Deep Well MagnaBot® II Magnetic Separation Device (Cat.# V3031)
- PolyATtract® System 1000 Magnetic Separation Stand (Cat.# Z5410)
- high-throughput *E. coli* purification buffer

Materials to Be Supplied by the User (continued)

(Solution compositions are provided in Section 7.C.)

- high-throughput *E. coli* purification buffer with 2mM ATP and 10mM $MgSO_4$ or 10mM $Mg(CH_3COO)_2$ (optional)
- HaloTEV Protease (Cat.# G6601)
- plate mixer
- 50ml conical tube
- 96-well assay blocks, V-bottom, 2ml, sterile (e.g., Costar Cat.# 3960)
- 96-well assay blocks, V-bottom, 500µl, sterile (e.g., Costar Cat.# 3956)
- Collection Plates (4-pack) (Cat.# A9161)
- liquid-handling platform or multichannel pipette
- SDS-polyacrylamide gel and gel apparatus

HaloTag® Fusion Protein Induction With Single Step (KRX) Cells

1. For each HaloTag® fusion protein, inoculate 200µl of LB medium that contains the appropriate antibiotic with Single Step KRX cells expressing the protein of interest.
2. Culture cells overnight at 37°C with shaking.
3. Add 20µl of overnight culture to 1ml of late auto-induction medium in a 2ml V-bottom 96-well assay block.
4. Culture cells for 21–24 hours at 25°C with shaking.

Cell Lysis

5. Centrifuge cells at 5,000 × *g* for 5 minutes. Remove and discard medium.
6. Resuspend each cell pellet with 125µl of *E. coli* lysis buffer.
7. Transfer lysates using a liquid-handling platform or a multichannel pipette to a 500µl V-bottom 96-well assay block, and mix vigorously for 30 minutes at room temperature (22–25°C).

Magne® HaloTag® Beads Equilibration

8. Resuspend Magne® HaloTag® Beads thoroughly by inverting the bottle. Transfer 5.5ml of Magne® HaloTag® Beads slurry to a 50ml conical tube. The ratio of bead slurry to bacterial culture is 50µl of slurry per 1ml of culture.
9. Place tube in the magnetic stand for 30 seconds to capture the beads. Carefully remove the supernatant and discard.
10. Remove tube from the magnetic stand, and add 42ml of high-throughput *E. coli* purification buffer. Mix thoroughly. Place tube in the magnetic stand for 30 seconds. Carefully remove the buffer and discard.
11. Repeat Step 10 twice for a total of three washes. After the last wash, do not remove the buffer.

HaloTag® Fusion Protein Binding

12. Pipet beads up and down to obtain a uniform slurry. Transfer 375µl of equilibrated beads to each well of cell lysate. Mix vigorously to maintain the beads in suspension for 1 hour.

4.A. Automated and Manual Purification of HaloTag® Fusion Proteins from *E. coli* in a 96-Well Plate (continued)

Washing

13. To remove the unbound proteins, place the plate on the Deep Well MagnaBot® II Magnetic Separation Device for 30 seconds. Remove the supernatant, and save for analysis if desired (see Section 4.D).
14. Wash Magne® HaloTag® Beads as follows:
    a. Add 400µl of high-throughput *E. coli* purification buffer per well, and mix vigorously for 10 minutes; if necessary, pipet up and down to resuspend the beads. Place plate on the magnetic stand for 30 seconds. Remove and discard the supernatant.
    b. Remove plate from the magnetic stand, and add 300µl of high-throughput *E. coli* purification buffer per well. Mix vigorously for 10 minutes. Place plate on the magnetic stand for 30 seconds. Remove and discard the supernatant.
    c. Remove plate from the magnetic stand, and add 200µl of high-throughput *E. coli* purification buffer per well. Mix vigorously for 10 minutes. Place plate on the magnetic stand for 30 seconds. Remove and discard the supernatant.

       Optional: Include 2mM ATP and 10mM $MgSO_4$ or 10mM $Mg(CH_3COO)_2$ in the high-throughput *E. coli* purification buffer used in this wash step, and increase the mixing time to 30 minutes to reduce copurification of *E. coli* chaperonins.
    d. Remove plate from the magnetic stand, and add 200µl of high-throughput *E. coli* purification buffer per well. Mix vigorously for 10 minutes. Place plate on the magnetic stand for 30 seconds. Remove and discard the supernatant.
    e. Remove plate from the magnetic stand, and add 100µl of high-throughput *E. coli* purification buffer per well. Mix vigorously for 10 minutes. Place plate on the magnetic stand for 30 seconds. Carefully remove the supernatant and discard.

Proteolytic Cleavage

15. Combine 11ml of high-throughput *E. coli* purification buffer and 66µl of HaloTEV Protease to prepare the cleavage buffer.
16. Add 100µl of cleavage buffer to each well, and mix vigorously for 90 minutes.

Protein Elution

17. Place plate in the magnetic stand for 30 seconds. Transfer the cleavage buffer to a 96-well Collection Plate. This is the first elution.
18. Add 100µl of high-throughput *E. coli* purification buffer to the beads, and mix vigorously for 30 minutes.
19. Place plate in the magnetic stand for 30 seconds. Transfer the high-throughput *E. coli* purification buffer to the Collection Plate. This is the second elution.

    Note: If you require a more concentrated protein sample, keep the two eluted samples separate; otherwise, combine eluted samples from Steps 17 and 19.
20. Analyze eluted proteins by SDS polyacrylamide gel electrophoresis (SDS-PAGE).

4.B. Purification of HaloTag® Fusion Proteins from *E. coli* (50ml of Culture)

The volumes of Magne® HaloTag® Beads and HaloTEV Protease can be scaled up or down as appropriate for the volume of bacterial culture.

Optional: Treatment with 2mM ATP and 10mM magnesium sulfate ($MgSO_4$) or magnesium acetate [$Mg(CH_3COO)_2$] can reduce copurification of *E. coli* chaperonins (e.g., DnaK [70kDa], DnaJ [37kDa], GrpE [40kDa], GroEL [57kDa] and GroES [10kDa] (2). Incubate the cell lysate in high-throughput *E. coli* purification buffer with 2mM ATP and 10mM $MgSO_4$ or 10mM $Mg(CH_3COO)_2$ for 10 minutes at 37°C prior to protein purification, or incubate the Magne® HaloTag® Beads in high-throughput *E. coli* purification buffer with 2mM ATP and 10mM $MgSO_4$ or $Mg(CH_3COO)_2$ for 30 minutes during one of the wash steps, followed by a high-throughput *E. coli* purification buffer wash.

During mixing steps, the shaking speed must be sufficient to maintain the beads in suspension. If the beads are allowed to settle or dry, binding, washing and proteolytic cleavage efficiencies will be reduced.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 7.C.)

- *E. coli* expressing the HaloTag® fusion protein of interest
- LB medium containing the appropriate antibiotic
  (**Note:** When expressing proteins that are toxic to cells, add 0.4% glucose to the LB medium.)
- late or early auto-induction medium
- cell sonicator
- PolyATtract® System 1000 Magnetic Separation Stand (Cat.# Z5410)
- HaloTag® protein purification buffer
- rotating platform or orbital shaker
- HaloTEV Protease (Cat.# G6601)
- SDS-polyacrylamide gel and gel apparatus

Preparation of Bacterial Expression Culture

1. Inoculate 2ml of LB medium that contains the appropriate antibiotic with *E. coli* expressing the HaloTag® fusion protein of interest. Culture overnight at 37°C with shaking.

   Note: If the protein is toxic to the cells, add 0.4% glucose to the medium.

2. Add 0.5ml of overnight culture to 49.5ml of late or early auto-induction medium. Incubate cultures with shaking to the desired optical density as follows:

Auto-Induction Medium	Approximate Induction Optical Density (OD_{600})	Growth Temperature	Growth Time
Late auto-induction	1.0–1.2	25°C	24 hours
Early auto-induction	0.6–0.8	25°C	16–18 hours

4.B. Purification of HaloTag® Fusion Proteins from *E. coli* (50ml of Culture) (continued)

Cell Lysis

This protocol is for 50ml of cells grown in LB medium. Optimal sonication conditions must be determined empirically. Avoid excessive sonication because this will denature the HaloTag® protein, preventing it from binding to the Magne® HaloTag® Beads.

3. If desired, remove and reserve 100µl of bacterial culture to analyze protein expression and solubility (see Section 4.D). Centrifuge the remaining volume at 4,000 × *g* for 20 minutes at 4°C, remove the medium and resuspend in 5ml of HaloTag® protein purification buffer.
4. Sonicate cells on ice, alternating 5-second bursts with 5-second cooling time at a recommend power output of 6–9W for a total of 2 minutes.

   Overheating will inhibit binding of the HaloTag® fusion protein.

Note: Reserve 100µl of the total protein fraction to analyze protein expression and solubility, if desired (see Section 4.D).

5. Centrifuge at 10,000 × *g* for 30 minutes at 4°C. Transfer the supernatant, which represents the soluble protein fraction, to a new tube.

   Note: Reserve 100µl of the soluble protein fraction to analyze protein expression and solubility, if desired (see Section 4.D).

Magne® HaloTag® Beads Equilibration

6. Resuspend Magne® HaloTag® Beads thoroughly by inverting the bottle. Transfer 2.5ml of Magne® HaloTag® Beads slurry to a 50ml conical tube.
7. Place tube on the magnetic stand for 30 seconds to capture the beads. Carefully remove the supernatant and discard.
8. Remove tube from the magnetic stand, and add 10ml of HaloTag® protein purification buffer. Mix thoroughly for 2 minutes.
9. Place tube on the magnetic stand for 30 seconds. Carefully remove the supernatant and discard.
10. Repeat Steps 8 and 9 three times for a total of four washes.

HaloTag® Fusion Protein Binding

11. Add the cell lysate prepared in Step 5 to the equilibrated beads.
12. Incubate for 1 hour at room temperature (22–25°C) with constant mixing. Make sure beads remain in suspension.
13. Place tube on the magnetic stand for 30 seconds. Carefully remove and reserve the supernatant, which represents the flowthrough, for analysis if desired (see Section 4.D).

Washing

14. Remove tube from the magnetic stand, and add 10ml of HaloTag® protein purification buffer. Mix thoroughly for 5 minutes.
15. Place tube on a magnetic stand for 30 seconds. Remove and discard the supernatant.
16. Repeat Steps 14 and 15 twice for a total of three washes.

Proteolytic Cleavage

17. Combine 30µl of HaloTEV Protease and 970µl of HaloTag® protein purification buffer to prepare the cleavage buffer.
18. Add cleavage buffer to the beads, and incubate at room temperature for 90 minutes with constant mixing.

Protein Elution

19. Place tube on a magnetic stand for 30 seconds. Remove and reserve the supernatant, which contains the cleaved protein. This is the first elution.
20. Remove tube from the magnetic stand, and add 1ml of HaloTag® protein purification buffer to the beads. Mix thoroughly for 15 minutes.
21. Place tube on the magnetic stand for 30 seconds. Remove and reserve the supernatant. This is the second elution.

    Note: If you require a more concentrated protein sample, keep the two eluted samples separate; otherwise, combine eluted samples from Steps 19 and 21.
22. Analyze the eluted proteins by SDS-PAGE.

4.C. Purification of HaloTag® Fusion Proteins from Mammalian Cells

This protocol describes protein purification from 1–3 × 10^8 mammalian cells. The volumes of Magne® HaloTag® Beads and HaloTEV Protease can be scaled up or down as appropriate for the number of mammalian cells. See Table 2.

Table 2. Recommended Reagent Volumes for Purification of HaloTag® Fusion Proteins from Mammalian Cells.

Material	T150 Flask (40ml)	Spinner Flask (120ml)
HEK293 or HEK293T cells	2–6 × 10^7 cells	1–3 × 10^8 cells
HaloTag® protein purification buffer or detergent-containing lysis buffer[1]	1ml	5ml
Protease Inhibitor Cocktail, 50X	20µl	100µl
Magne® HaloTag® Beads	125µl slurry (25µl settled beads)	375µl slurry (75µl settled beads)
HaloTEV Protease	1.5µl (7.5 units)	4.5µl (22.5 units)
cleavage buffer (elution volume)	50µl	150µl

[1]Other lysis buffers and methods are described in the *HaloTag® Mammalian Protein Detection and Purification Systems Technical Manual* #TM348.

4.C. Purification of HaloTag® Fusion Proteins from Mammalian Cells (continued)

During mixing steps, the shaking speed must be sufficient to maintain the beads in suspension. If the beads are allowed to settle or dry, binding, washing and proteolytic cleavage efficiencies will be reduced.

Materials to Be Supplied by the User

(Solution compositions are provided in Section 7.C.)

- 1–3 × 10^8 mammalian cells expressing the HaloTag® fusion protein of interest, harvested, centrifuged to form a cell pellet and medium removed
- Protease Inhibitor Cocktail, 50X (Cat.# G6521)
- HaloTag® protein purification buffer
- cell sonicator
- PolyATtract® System 1000 Magnetic Separation Stand (Cat.# Z5410)
- rotating platform or orbital shaker
- HaloTEV Protease (Cat.# G6601)
- SDS-polyacrylamide gel and gel apparatus

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

Cell Lysis

2. Resuspend the cell pellet in 5ml of HaloTag® protein purification buffer.
3. Add 100µl of Protease Inhibitor Cocktail, 50X.
4. Sonicate cells on ice using 10-second bursts with 10-second cooling time between each cycle for a total of 1 minute at power output of 3–6W.

Overheating will inhibit binding of the HaloTag® fusion protein.

5. Centrifuge the cell lysate for 15 minutes at 10,000 × *g* at 4°C, and reserve the supernatant.

Magne® HaloTag® Beads Equilibration

6. Resuspend Magne® HaloTag® Beads thoroughly by inverting the bottle. Transfer 375µl of Magne® HaloTag® Beads slurry to a 15ml conical tube.
7. Place tube on the magnetic stand for 30 seconds to capture the beads. Carefully remove the supernatant and discard.
8. Remove tube from the magnetic stand, and add 5ml of HaloTag® protein purification buffer. Mix thoroughly for 5 minutes.
9. Place tube on the magnetic stand for 30 seconds. Carefully remove the supernatant and discard.
10. Repeat Steps 8 and 9 three times for a total of four washes.

HaloTag® Fusion Protein Binding

11. Add the cell lysate prepared in Step 5 to the equilibrated beads.
12. Incubate for 90 minutes at room temperature (22–25°C) with constant mixing.
13. Place tube on the magnetic stand for 30 seconds. Carefully remove the supernatant, and reserve this flowthrough to analyze protein expression levels and binding efficiency if desired (see Section 4.D).

Washing

14. Remove tube from the magnetic stand, and add 5ml of HaloTag® protein purification buffer. Mix thoroughly for 10 minutes.
15. Place tube on a magnetic stand for 30 seconds. Remove and discard the supernatant.
16. Repeat Steps 14 and 15 twice for a total of three washes.

Proteolytic Cleavage

17. Combine 4.5µl of HaloTEV Protease and 145.5µl of HaloTag® protein purification buffer to prepare the cleavage buffer.
18. Add cleavage buffer to the beads, and incubate at room temperature for 90 minutes with constant mixing.

Protein Elution

19. Place tube on a magnetic stand for 30 seconds. Carefully remove and reserve the supernatant, which contains the cleaved protein. This is the first elution.
20. Remove tube from the magnetic stand, and add 150µl of HaloTag® protein purification buffer to the beads. Mix thoroughly for 30 minutes.
21. Place tube on a magnetic stand for 30 seconds. Carefully remove and reserve the supernatant. This is the second elution.

    Note: If you require a more concentrated protein sample, keep the two eluted samples separate; otherwise, combine eluted samples from Steps 19 and 21.
22. Analyze eluted proteins by SDS-PAGE.

Promega

4.D. Detection of HaloTag® Fusion Proteins Expressed in *E. coli* and Mammalian Cells (Optional)

The covalent bond between the HaloTag® fusion protein and HaloTag® ligand allows analysis by SDS-PAGE and detection of the fluorescently labeled HaloTag® fusion protein using a fluorescent scanner. You can evaluate the protein expression level, solubility and binding efficiency by labeling the HaloTag® fusion protein with a HaloTag® ligand. You can assess the expression level by labeling an aliquot of the sample after sonication, solubility by labeling an aliquot of the sample after centrifugation, and binding efficiency by labeling an aliquot of the sample after binding (i.e., the flowthrough) to help determine the outcome of protein purification.

This protocol uses the HaloTag® TMR Ligand to label the HaloTag® fusion protein. For a complete list of available fluorescent HaloTag® ligands, see Section 7.D. For additional information about HaloTag® labeling refer to the *HaloTag® Technology: Focus on Imaging Technical Manual* #TM260.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 7.C.)

- HaloTag® TMR Ligand (Cat.# G8251)
- 100% DMSO, 1X PBS (pH 7.5) or HaloTag® protein purification buffer
- 4X SDS gel loading buffer
- water bath or heat block set at 70°C
- SDS polyacrylamide gel and gel apparatus
- fluorescent scanner with an excitation wavelength of 555nm and an emission wavelength of 585nm, such as the Typhoon® imager (GE Healthcare Biosciences)
- Coomassie® blue stain

1. Dilute the HaloTag® TMR Ligand (5mM) 100-fold in DMSO to make a 50µM working solution, which can be stored, protected from light, at –20°C. Alternatively, the working solution can be prepared in 1X PBS or HaloTag® protein purification buffer but should be used the same day. Do not store the ligand diluted in PBS or HaloTag® protein purification buffer.
2. Combine 20µl of reserved material (starting material, total protein fraction, soluble protein fraction or flowthrough), 1µl of 50µM HaloTag® TMR Ligand and 9µl of HaloTag® protein purification buffer in a 1.5ml microcentrifuge tube.
3. Incubate tubes for 20 minutes at room temperature (22–25°C).
4. Add 10µl of 4X SDS gel loading buffer to each tube, and incubate for 2 minutes at 70°C.
5. Analyze 10–20µl of each sample by SDS-PAGE.
6. Scan the gel using a fluorescent scanner with an excitation wavelength of 555nm and an emission wavelength of 585nm, then stain the gel with Coomassie® blue stain. To examine protein solubility compare the amount of the protein of interest in the total and soluble fractions.

5. HaloTag® Protein Pull-Down

Protein pull-down from cell-free expression systems using the Magne® HaloTag® Beads can be used to confirm protein interactions and map protein domains. To use this protocol, the bait protein must contain the HaloTag® protein on the C- or N-terminus. The potential binding partner (prey) can be protein, DNA or RNA, but you must have some way of detecting the prey (e.g., Western blot analysis or incorporation of a fluorescence or biotin label). Prey proteins can be labeled co-translationally with radioactivity (e.g., ^{35}S-methionine), BODIPY® dye (FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System, Cat.# L5001) or biotin (Transcend™ Colorimetric Translation Detection System, Cat.# L5070).

Both bait and prey proteins can be expressed in cell-free expression systems following the protocol in the appropriate cell-free expression system technical manual. HaloTag® protein pull-down assays are compatible with Promega cell-free expression systems such as those listed in Table 3.

Typically, the bait is bound to the beads, then introduced to a pool of proteins containing prey protein (Figure 2). Alternatively, bait and prey proteins can associate in solution, and the complex can be captured using the Magne® HaloTag® Beads, which bind the bait fusion protein. Specific and covalent binding of bait to the beads enables efficient bait capture even at low expression levels and minimizes bait loss during incubation with prey.

Table 3. Cell-Free Expression Systems for Expression of Bait and Prey Proteins.

Cell-Free Expression System	Volume of Magne® HaloTag® Beads Slurry per 50µl Cell-Free Expression Reaction
TnT® T7 Quick Coupled Transcription/Translation System (Cat.# L1170)[1]	5µl
TnT® SP6 Quick Coupled Transcription/Translation System (Cat.# L2080)[1]	5µl
TnT® SP6 High-Yield Wheat Germ Protein Expression System (Cat.# L3260)[2]	7.5µl
S30 T7 High-Yield Protein Expression System (Cat.# L1110)[3]	7.5µl

[1]For more information and a protocol for cell-free protein expression, see the *TnT® Quick Coupled Transcription/Translation System Technical Manual* #TM045.

[2]For more information and a protocol for cell-free protein expression, see the *TnT® SP6 High-Yield Wheat Germ Protein Expression System Technical Manual* #TM282.

[3]For more information and a protocol for cell-free protein expression, see the *S30 T7 High-Yield Protein Expression System Technical Manual* #TM306.

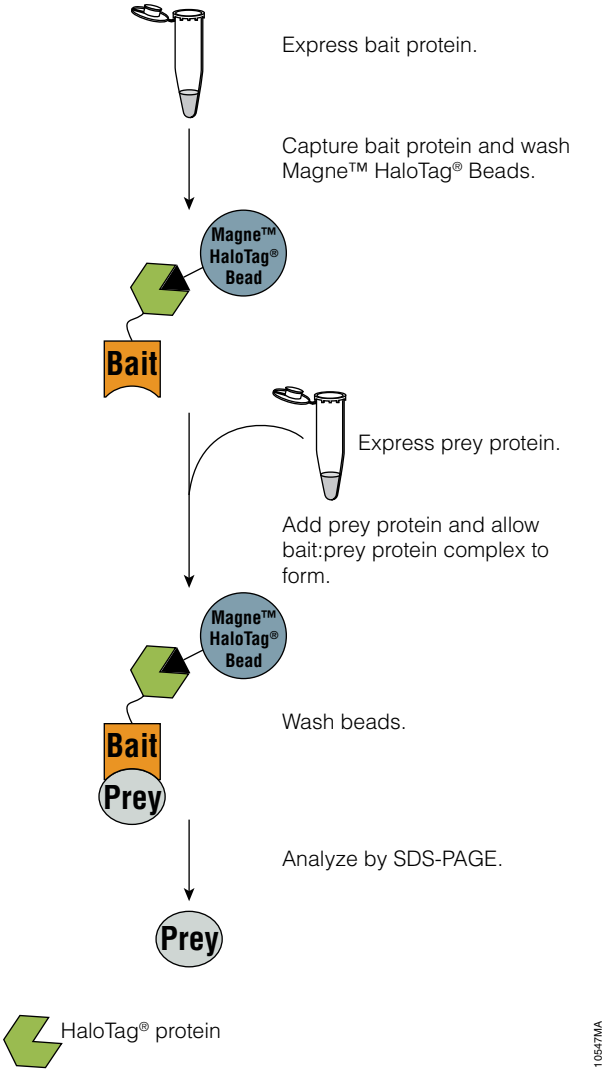

Figure 2. Schematic diagram of the HaloTag® protein pull-down assay.

5.A. Protein Pull-Down Protocol from a Cell-Free Expression System

Protein pull-downs may require optimization.

The pH range for HaloTag® fusion protein binding is pH 6.5–8.0. The recommended NaCl concentration range is 50–150mM. We recommend using one of the following equilibration buffers:

- TBS (Tris-buffered saline) with 0.005% IGEPAL® CA-630
- PBS (phosphate-buffered saline) with 0.005% IGEPAL® CA-630
- 20mM HEPES (pH 7.6), 150mM NaCl, 0.005% IGEPAL® CA-630

In this protocol, bait and prey binding steps are sequential. However, the bait and prey complex can be formed prior to protein pull-down.

This protocol can be scaled up or down.

! During mixing steps, the shaking speed must be sufficient to maintain the beads in suspension. If the beads are allowed to settle or dry, binding, washing and proteolytic cleavage efficiencies will be reduced.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 7.C.)

- MagneSphere® Technology Magnetic Separation Stand for 0.5ml tubes (two-position, Cat.# Z5331; twelve-position, Cat.# Z5341) or MagneSphere® Technology Magnetic Separation Stand for 1.5ml tubes (two-position, Cat.# Z5332; twelve-position, Cat.# Z5342)
- equilibration buffer
- rotating platform or orbital shaker
- cell-free expression reaction expressing bait protein
- cell-free expression reaction expressing prey protein
- 1X SDS gel loading buffer

Magne® HaloTag® Beads Equilibration

1. Resuspend Magne® HaloTag® Beads thoroughly by inverting the bottle. Calculate the required volume of Magne® HaloTag® Beads slurry (see Table 3). Add the appropriate volume of bead slurry to a 0.5ml or 1.5ml microcentrifuge tube.
2. Place tube in the magnetic stand for 30 seconds to capture the beads. Carefully remove the supernatant and discard.
3. Remove tube from the magnetic stand, and add 50µl of equilibration buffer. Mix thoroughly for 5 minutes.

5.A. Protein Pull-Down Protocol from a Cell-Free Expression System (continued)

Magne® HaloTag® Beads Equilibration (continued)

4. Place tube on a magnetic stand for 30 seconds. Carefully remove the supernatant and discard.
5. Repeat Steps 3 and 4 three times for a total of four washes. After the last wash, do not remove the supernatant until immediately before use in Step 6.

HaloTag® Fusion Protein (Bait) Binding

6. Add an equal volume of equilibration buffer to the cell-free expression reaction with the bait protein. Discard the supernatant from Step 5, and add the cell-free reaction expressing the bait protein to the equilibrated beads.

   Note: We recommend reserving 1µl of the cell-free reaction to evaluate expression levels in the starting material via labeling with the HaloTag® TMR Ligand (see Section 5.C).
7. Mix thoroughly for 1 hour at room temperature or for 4–5 hours at 4°C. Make sure beads remain in suspension.
8. Place tube on the magnetic stand for 30 seconds. Carefully remove the supernatant.

   Note: This is the flowthrough fraction. We recommend reserving 1µl to analyze protein-binding efficiency via labeling with the HaloTag® TMR Ligand (see Section 5.C).
9. Remove tube from the magnetic stand, and add 50µl of equilibration buffer. Mix thoroughly for 5 minutes.
10. Place tube on the magnetic stand for 30 seconds. Carefully remove the supernatant and discard.
11. Repeat Steps 9 and 10 twice for a total of three washes.

Prey Binding

12. Add an equal volume of equilibration buffer to the cell-free expression reaction with the prey protein. Add the cell-free reaction expressing the prey protein to the beads.

    Note: Typically, the volume of cell-free reaction expressing prey protein is equal to the volume of cell-free reaction expressing bait protein added in Step 6.
13. Mix thoroughly for 1 hour at room temperature or for 4–5 hours at 4°C. Make sure beads remain in suspension.
14. Place tube on the magnetic stand for 30 seconds. Carefully remove the supernatant and discard.
15. Remove tube from the magnetic stand, and add 50µl of equilibration buffer. Mix thoroughly for 1–2 minutes.
16. Place tube on the magnetic stand for 30 seconds. Carefully remove the supernatant and discard.
17. Repeat Steps 15 and 16 for a total of two or three washes.
18. Add 20µl of 1X SDS gel loading buffer to the beads, and analyze by SDS-PAGE or Western blot analysis to detect the prey protein.

5.B. HaloTag® Mammalian Pull-Down Protocol

The Magne® HaloTag® Beads can be used in place of nonmagnetic HaloLink™ Resin in the HaloTag® Mammalian Pull-Down System (Cat.# G6504), HaloTag® Mammalian Pull-Down and Labeling System (Cat.# G6500) and HaloTag® Complete Pull-Down System (Cat.# G6509). Follow the protocols described in the *HaloTag® Mammalian Pull-Down and Labeling Systems Technical Manual* #TM342 and *HaloTag® Complete Pull-Down System Technical Manual* #TM360 and make the following modifications:

- Use 100µl of Magne® HaloTag® Beads instead of 200µl of the nonmagnetic HaloLink™ Resin listed in the protocols. Other reagent volumes, incubation times and temperatures should remain unchanged.
- Perform TEV protease cleavage to elute the captured proteins; do not use SDS gel loading buffer. Eluting proteins with SDS gel loading buffer instead of TEV protease will release captured proteins as well but may result in increased background.

5.C. Detection of HaloTag® Fusion Proteins Expressed in a Cell-Free Expression System (Optional)

Fluorescent labeling of the HaloTag® fusion protein with the HaloTag® TMR Ligand provides a rapid and convenient method to monitor protein expression and determine binding efficiency of the bait protein. The covalent bond between the HaloTag® fusion protein and HaloTag® ligand allows resolution by SDS-PAGE and detection of the fluorescently labeled fusion proteins using a fluorescent scanner.

This protocol uses the HaloTag® TMR Ligand to label the HaloTag® fusion protein. For a complete list of available fluorescent HaloTag® ligands, see Section 7.D. For additional information about HaloTag® labeling, refer to the *HaloTag® Technology: Focus on Imaging Technical Manual* #TM260.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 7.C.)

- HaloTag® TMR Ligand (Cat.# G8251)
- 100% DMSO, 1X PBS or HaloTag® protein purification buffer
- cell-free expression reaction expressing the HaloTag® fusion protein of interest
- equilibration buffer (see Section 5.A)
- 1X SDS gel loading buffer
- water bath or heat block set at 70°C
- SDS-polyacrylamide gel and gel apparatus
- fluorescent scanner with an excitation wavelength of 555nm and an emission wavelength of 585nm, such as the Typhoon® instrument (GE Healthcare Biosciences)

1. Dilute the HaloTag® TMR Ligand (5mM) 100-fold in DMSO to make a 50µM working solution, which can be stored, protected from light, at –20°C. Alternatively, the working solution can be prepared in 1X PBS or HaloTag® protein purification buffer but should be used the same day. Do not store the ligand diluted in PBS or HaloTag® protein purification buffer.

5.C. Detection of HaloTag® Fusion Proteins Expressed in a Cell-Free Expression System (Optional; continued)

2. To analyze HaloTag® fusion protein expression levels in the starting material, combine 1µl of the cell-free expression reaction with 5µl of equilibration buffer and 1µl of 50µM HaloTag® TMR Ligand.

   To analyze the binding efficiency, combine 1µl of flowthrough from Section 5.A with 5µl of equilibration buffer and 1µl of 50µM HaloTag® TMR Ligand.

3. Incubate tubes at room temperature for 15–30 minutes, protected from light.
4. Add 15µl of 1X SDS gel loading buffer, and heat at 70°C for 3 minutes.
5. Analyze samples by SDS-PAGE.
6. Scan the gel on a fluorescent scanner with an excitation wavelength of 555nm and an emission wavelength of 585nm, and quantitate band intensities.

6. Troubleshooting

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

6.A. HaloTag® Protein Purification

For additional troubleshooting information about expression and detection of HaloTag® fusion proteins in mammalian systems, see the *HaloTag® Mammalian Protein Detection and Purification Systems Technical Manual* #TM348. For additional troubleshooting information about expression and detection of HaloTag® fusion proteins in *E. coli*, see the *HaloTag® Protein Purification System Technical Manual* #TM312.

Symptoms	Causes and Comments
Inefficient protein binding	Magne® HaloTag® Beads were allowed to settle or dry during protein binding. Increase the mixing speed to maintain beads in suspension. Do not allow beads to dry.
	Magne® HaloTag® Beads were not equilibrated properly. Equilibrate the beads thoroughly with high-throughput *E. coli* purification buffer (Section 4.A) or HaloTag® protein purification buffer (Sections 4.B and 4.C).
	Samples were sonicated too long or became overheated during sonication. Decrease sonication time and output settings. Keep samples on ice during sonication.
	Avoid protease inhibitor cocktails that contain AEBSF, especially when working with proteins expressed at low levels or in mammalian cells. AEBSF interferes with HaloTag® binding.

Symptoms	Causes and Comments
Inefficient protein binding (continued)	Improper binding conditions. • Protein expression level was high, and the beads were saturated. Increase the amount of beads. • Binding time was not sufficient. Increase binding time, such as overnight at 4°C. • Binding conditions were not optimal. The optimal pH range is pH 6.5–8.0. Check pH of the lysate if necessary. • Target protein formed a multimer. Dilute the cell lysate prior to protein binding. • The structure of the target protein interfered with the HaloTag® protein reactive site. Alter the directionality of the fusion.
Inefficient HaloTEV Protease cleavage	Magne® HaloTag® Beads were allowed to settle or dry during protease cleavage. Increase the mixing speed to maintain beads in suspension. Do not allow beads to dry.
	Increase the incubation time for the HaloTEV Protease cleavage, such as overnight at 4°C.
	Increase the amount of HaloTEV Protease in the cleavage reaction.
	Additives in the HaloTEV Protease cleavage reaction inhibited HaloTEV Protease activity. For more information, see the *HaloTag® Mammalian Protein Detection and Purification Systems Technical Manual* #TM348.
Fusion protein retained by the Magne® HaloTag® Beads	The HaloTag® protein is negatively charged at pH 7.5. Proteins that are positively charged at this pH interacted with the HaloTag® protein through ionic interaction. Increase the ionic strength of the elution buffer by adding NaCl (up to a final concentration of 500mM).
	Hydrophobic proteins interacted nonspecifically with Magne® HaloTag® Beads. They also may precipitate under high salt conditions. Using a nonionic detergent (i.e., IGEPAL® CA-630 at a final concentration of 0.005–0.05%) during elution may enhance protein recovery.
	Increase elution time. Add HaloTag® protein purification buffer to the beads, and incubate at 4°C overnight to release the protein.

6.A. HaloTag® Protein Purification (continued)

Symptoms	Causes and Comments
Multiple bands or contaminant proteins co-eluted with target protein of interest	Co-elution of HaloTEV Protease (81.5kDa) due to saturation of beads. To remove contaminating HaloTEV Protease, add additional equilibrated Magne® HaloTag® Beads at the elution step and incubate for 30 minutes with constant mixing to bind and remove HaloTEV Protease.
	Protein was degraded. Add Protease Inhibitor Cocktail (Cat.# G6521) to the buffer used to wash the beads and, if necessary, to the final eluted sample at a final concentration of 1X. Perform all protein purification steps at 4°C.
	Check for premature stop codons in the protein-coding sequence. Eliminate them if possible.
	Additives in the binding step, such as glycerol or salt and certain detergents at high concentrations, can promote nonspecific adsorption or aggregation of proteins onto the Magne® HaloTag® Beads. See Table 4. Avoid these additives if possible.
	Add freshly prepared IGEPAL® CA-630 to the HaloTag® protein purification buffer at a final concentration of 0.005% to reduce nonspecific binding to the beads.
	The amount of Magne® HaloTag® Beads used for protein capture was too high. Reduce the amount of Magne® HaloTag® Beads used.
	Increase the wash volume and time.
	Increase wash stringency by increasing the salt concentration or adding detergents to the buffer used to wash the Magne® HaloTag® Beads. Do not use high-salt conditions and high detergent concentrations at the same time.
	Use different wash conditions, such as high-salt washes followed by low-salt washes.
	Excessive sonication led to nonspecific protein binding to the beads. Optimize sonication conditions, or use milder cell lysis methods.
	Excessive sonication or improper cell lysis conditions led to denaturation or aggregation of target protein. Use proper cell lysis conditions for optimal protein recovery.

Symptoms	Causes and Comments
Multiple bands or contaminant proteins co-eluted with target protein of interest (continued)	Add reducing agent such as dithiothreitol (DTT) to the buffer used to wash the Magne® HaloTag® Beads at a final concentration of 1mM to reduce disulfide bond-linked contaminants.
	Protein cofactors that are required for protein function or proper folding were co-purified.
	Tubulin (50kDa) was co-eluted when purifying protein from HEK293 or HEK293T cells. Prior to proteolytic cleavage, wash the beads for 30 minutes with a low-salt buffer (≤50mM NaCl).
	E. coli chaperonins copurified with the protein of interest. Treatment with 2mM ATP and 10mM $MgSO_4$ or 10mM $Mg(CH_3COO)_2$ can reduce copurification of *E. coli* chaperonins (see Section 4.A).

6.B. HaloTag® Protein Pull-Down

For additional troubleshooting information about cell-free protein expression in mammalian systems, see the *TnT® Quick Coupled Transcription/Translation System Technical Manual* #TM045, *TnT® SP6 High-Yield Wheat Germ Protein Expression System Technical Manual* #TM282 or *S30 T7 High-Yield Protein Expression System Technical Manual* #TM306.

Symptoms	Causes and Comments
Inefficient bait binding	Magne® HaloTag® Beads were allowed to settle or dry during protein binding. Increase the mixing speed to maintain beads in suspension. Do not allow beads to dry.
	Magne® HaloTag® Beads were not equilibrated properly. Equilibrate the beads thoroughly with equilibration buffer.
	Improper binding conditions. • Binding time was not sufficient. Increase binding time, such as overnight at 4°C. • Binding conditions were not optimal. The optimal pH range is pH 6.5–8.0. Check pH of the lysate if necessary. • Target protein formed a multimer. Dilute the lysate prior to protein binding. • The structure of the target protein interfered with the HaloTag® protein reactive site. Alter the directionality of the fusion.
	Avoid protease inhibitor cocktails that contain AEBSF, especially when working with proteins expressed at low levels or in mammalian cells. AEBSF interferes with HaloTag® binding.

6.B. HaloTag® Protein Pull-Down (continued)

Symptoms	Causes and Comments
Excessive background	Stringent washes may be needed prior to prey binding. Increase wash stringency by increasing the salt concentration or adding detergents to the buffer used to wash the Magne® HaloTag® Beads. Do not use high-salt conditions and high detergent concentrations at the same time.
	Use different wash conditions such as high-salt washes followed by low-salt washes.
	Increase the wash volume and time.
Inefficient prey binding	If you performed stringent washes prior to prey binding, ensure wash buffer removal by performing more washes with equilibration buffer.
	Make sure the bait was not denatured during handling or stringent washes.
	Interaction may require certain cofactors.

7. Appendix

7.A. General Considerations

The effects of common laboratory reagents on protein purification using the Magne® HaloTag® Beads are summarized in Table 4. For a particular protein of interest, the effect of these reagents may be different and should be determined empirically.

Table 4. The Effect of Common Reagents on HaloTag® Protein Binding.

Reagent	Comments	Effect
DTT	Use up to 50mM during protein binding.	DTT reduces background caused by intermolecular disulfide linkages and assists in HaloTEV Protease cleavage.
PMSF	Use up to 5mM in binding steps.	PMSF prevents proteolysis.
AEBSF	Avoid protease inhibitor cocktails that contain AEBSF, especially when working with proteins expressed at low levels or in mammalian cells.	AEBSF interferes with HaloTag® fusion protein binding at low expression levels, especially for mammalian expression.
urea/guanidine	Do not use.	Denaturants interfere with binding of HaloTag® fusion proteins, which requires native, folded protein.
Tween®-20	Use up to 0.1%.	OK for all steps.
Tween®-8	Use up to 0.1%.	OK for all steps.
OTG CHAPS OG Brij35 Triton® X-114 Triton® X-100 NP-40 (IGEPAL® CA-630)	Use up to 0.01%. Use up to 0.01%. Use up to 0.01%. Use up to 0.05%. Use up to 0.05%. Use up to 0.05%. Use up to 0.05%.	High concentrations may promote nonspecific adsorption to Magne® HaloTag® Beads and reduce the amount of protein eluted. The effect may be protein-dependent and may need to be determined empirically.

7.B. References

1. Ohana, R.F. (2009) HaloTag7: A genetically engineered tag that enhances bacterial expression of soluble proteins and improves protein purification. *Protein Expr. Purif.* **68**, 110–20.
2. Yu-Sherman, M. and Goldberg, A.L. (1992) Involvement of the chaperonin dnaK in the rapid degradation of a mutant protein in *Escherichia coli*. *EMBO J.* **11**, 71–7.

7.C. Composition of Buffers and Solutions

auto-induction medium (early)

LB medium containing 0.05% glucose and 0.05% rhamnose

Add filtered glucose and rhamnose solutions after autoclaving LB medium.

auto-induction medium (late)

LB medium containing 0.15% glucose and 0.2% rhamnose

Add filtered glucose and rhamnose solutions after autoclaving LB medium.

***E. coli* lysis buffer**

1X	FastBreak™ Cell Lysis Reagent (Cat.# V8571)
2mg/ml	lysozyme
20 units/ml	RQ1 RNase-Free DNase (Cat.# M6101)

Optional: 50X Protease Inhibitor Cocktail (Cat.# G6521) at a final concentration of 1X

glucose, 20% (w/v)

20g	D-glucose

Add distilled water to 100ml, filter through a 0.2µm filter and store in aliquots at –20°C.

HaloTag® protein purification buffer

50mM	HEPES (pH 7.5)
150mM	NaCl
1mM	DTT
0.005%	IGEPAL® CA-630

Note: Tris-buffered saline or phosphate-buffered saline can be substituted for the 50mM HEPES (pH 7.5) and 150mM NaCl.

high-throughput *E. coli* purification buffer

50mM	HEPES (pH 7.5)
150mM	NaCl
1mM	DTT
0.01%	IGEPAL® CA-630

LB medium

10g/L	Bacto®-tryptone
5g/L	Bacto®-yeast extract
5g/L	NaCl

Adjust the pH to 7.5 with NaOH. Autoclave.

1X PBS (pH 7.5)

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

phosphate-buffered saline (pH 7.4)

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

rhamnose, 20% (w/v)

10g	L-rhamnose monohydrate

Add distilled water to 45ml, filter through a 0.2µm filter and store in aliquots at –20°C.

7.C. Composition of Buffers and Solutions (continued)

1X SDS gel loading buffer

50mM	Tris-HCl (pH 6.8)
2%	SDS
0.1%	bromophenol blue
10%	glycerol
100mM	dithiothreitol

1X SDS gel loading buffer lacking dithiothreitol can be stored at room temperature. Dithiothreitol should be added from a 1M stock just before the buffer is used.

4X SDS gel loading buffer

0.24M	Tris-HCl (pH 6.8)
3mM	bromophenol blue
40%	glycerol
0.4M	dithiothreitol
8%	SDS

4X SDS gel loading buffer lacking dithiothreitol can be stored at room temperature. Dithiothreitol should be added from a 1M stock just before the buffer is used.

Tris-buffered saline (pH 7.5)

100mM	Tris buffer (pH 7.5)
150mM	NaCl

7.D. Related Products

Product	Size	Cat.#
HaloTEV Protease	200u	G6601
	800u	G6602
Protease Inhibitor Cocktail, 50X	1ml	G6521
Collection Plates (4-pack)	1 each	A9161
FastBreak™ Cell Lysis Reagent, 10X	15ml	V8571
	60ml	V8572
	100ml	V8573
RQ1 RNase-Free DNase	1,000u	M6101
Single Step (KRX) Competent Cells	20 × 50µl	L3002
HaloLink™ Resin	1.25ml	G1912
	2.5ml	G1913
	10ml	G1914
	25ml	G1915

7.D. Related Products (continued)

HaloTag® Complete Systems

Product	Size	Cat.#
HaloTag® Complete Pull-Down System	1 each	G6509
HaloTag® Mammalian Pull-Down and Labeling System	24 reactions	G6500
HaloTag® Mammalian Pull-Down System	24 reactions	G6504
HaloTag® Mammalian Protein Purification System	1 each	G6790
HaloTag® Mammalian Protein Detection and Purification System	1 each	G6795
HaloTag® Protein Purification System	1 each	G6280
HaloCHIP™ System	20 reactions	G9410

Vectors for Expressing the HaloTag® Fusion Protein in *E. coli*

Product	Size	Cat.#
pFN18A HaloTag® T7 Flexi® Vector	20µg	G2751
pFN18K HaloTag® T7 Flexi® Vector	20µg	G2681

Vectors for Expressing the HaloTag® Fusion Protein in Cell-Free Expression Systems

Product	Size	Cat.#
pFN19A HaloTag® T7 SP6 Flexi® Vector	20µg	G1891
pFN19K HaloTag® T7 SP6 Flexi® Vector	20µg	G1841
pFC20A HaloTag® T7 SP6 Flexi® Vector	20µg	G1681
pFC20K HaloTag® T7 SP6 Flexi® Vector	20µg	G1691

Vectors for Expressing the HaloTag® Fusion Protein in Mammalian Systems

Product	Size	Cat.#
pFC14A HaloTag® CMV Flexi® Vector	20µg	G9651
pFC14K HaloTag® CMV Flexi® Vector	20µg	G9661
pFN21A HaloTag® CMV Flexi® Vector	20µg	G2821
pFN21K HaloTag® CMV Flexi® Vector	20µg	G2831
pHTN HaloTag® CMV-neo Vector	20µg	G7721
pHTC HaloTag® CMV-neo Vector	20µg	G7711
HaloTag® Flexi® Vectors—CMV Deletion Series Sample Pack	9 × 2µg	G3780

HaloTag® Fluorescent Ligands

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

HaloTag® Building Blocks and Antibodies

Product	Size	Cat.#
HaloTag® Iodoacetamide (O2) Ligand	5mg	P1681
HaloTag® Succinimidyl Ester (O2) Ligand	5mg	P1691
HaloTag® Amine (O2) Ligand	5mg	P6711
Anti-HaloTag® pAb	200µg	G9281
Anti-HaloTag® Monoclonal Antibody	200µg	G9211

Flexi® Cloning Systems

Product	Size	Cat.#
HaloTag® Cloning Starter System	1 each	G6050
Flexi® System, Entry/Transfer	5 entry and 20 transfer reactions	C8640
Flexi® System, Transfer	100 transfer reactions	C8820
Carboxy Flexi® System, Transfer	50 transfer reactions	C9320
10X Flexi® Enzyme Blend (SgfI & PmeI)	25µl	R1851
	100µl	R1852
Carboxy Flexi® Enzyme Blend (SgfI & EcoICRI)	50µl	R1901

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7.D. Related Products (continued)

TnT® Cell-Free Protein Expression Systems

Product	Size	Cat.#
TnT® T7 Quick Coupled Transcription/Translation System	5 reactions	L1171
	40 reactions	L1170
TnT® SP6 Quick Coupled Transcription/Translation System	5 reactions	L2081
	40 reactions	L2080
TnT® SP6 High-Yield Wheat Germ Protein Expression System	40 reactions	L3260
	10 reactions	L3261
TnT® T7 Coupled Reticulocyte Lysate System	40 reactions	L4610
TnT® SP6 Coupled Reticulocyte Lysate System	40 reactions	L4600
TnT® T3 Coupled Reticulocyte Lysate System	40 reactions	L4950
TnT® T7 Quick for PCR DNA	40 reactions	L5540
TnT® T7 Coupled Wheat Germ Extract System	40 reactions	L4140
TnT® SP6 Coupled Wheat Germ Extract System	40 reactions	L4130
S30 T7 High-Yield Protein Expression System	24 reactions	L1110

Detection Systems

Product	Size	Cat.#
FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System	40 reactions	L5001
Transcend™ Colorimetric Translation Detection System	30 reactions	L5070
Transcend™ Chemiluminescent Translation Detection System	30 reactions	L5080

Magnetic Stands

Product	Size	Cat.#
MagneSphere® Technology Magnetic Separation Stand (two-position)	0.5ml	Z5331
	1.5ml	Z5332
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	0.5ml	Z5341
	1.5ml	Z5342
PolyATtract® System 1000 Magnetic Separation Stand	1 each	Z5410
Deep Well MagnaBot® 96 Magnetic Separation Device	1 each	V3031

DNA Isolation Systems

Product	Size	Cat.#
PureYield™ Plasmid Miniprep System	100 preps	A1223
	250 preps	A1222
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495

8. Summary of Changes

The following changes were made to the 12/15 revision of this document:

1. The 100% IGEPAL buffer was removed from the list of equilibration buffers.
2. Patent and disclaimer statements were updated.
3. The document design was updated.