TECHNICAL MANUAL

HaloLink™ Resin

Instructions for Use of Products
G1912, G1913, G1914 and G1915

Revised 9/16
TM250

HaloLink™ Resin

All technical literature is available at: www.promega.com/protocols/
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1. Description

The HaloLink™ Resin[a,b] provides a method for covalent and oriented attachment of HaloTag® fusion proteins onto a solid surface. The resin consists of a HaloTag® ligand bound to Sepharose® beads that specifically and rapidly bind HaloTag® fusion proteins. HaloLink™ Resin has high binding capacity. Due to covalent linkage, HaloTag® fusion proteins cannot be eluted from the resin, allowing extensive washing to remove nonspecifically bound protein without the danger of eluting HaloTag® fusion proteins. The binding rate is rapid and equivalent to biotin-streptavidin interactions. The HaloLink™ Resin is based on the HaloTag® technology, a platform technology for labeling and immobilization of proteins in vivo and in vitro. The HaloTag® technology is based on the efficient formation of a covalent bond between the HaloTag® protein, a 34.1kDa mutated hydrolase, and a specific ligand in living cells, in solution or on a solid support.

- **Composition:** HaloTag® binding ligand is covalently attached to the surface of Sepharose® beads via a carbamide linkage using an eleven-atom linker.
- **Particle Size**: 45–165 microns.
- **Concentration:** HaloLink™ Resin is supplied as 25% slurry in 25% ethanol.
- **Binding Capacity:** One milliliter of settled resin binds >7mg of HaloTag® fusion protein. Note: One milliliter of settled resin corresponds to 4ml of the 25% slurry provided.

2. Product Components and Storage Conditions

PRODUCT	SIZE*	CAT.#
HaloLink™ Resin	**1.25ml**	**G1912**
	2.5ml	**G1913**
	10ml	**G1914**
	25ml	**G1915**

*Size reflects volume of settled resin.

Storage Conditions: Store the HaloLink™ Resin at 4°C. Do not freeze.

3. HaloLink™ Resin Applications

The HaloLink™ Resin can be used in a variety of applications including detection of protein:protein interactions and purification of fusion proteins using protease cleavage. Due to the covalent linkage, HaloTag® fusion proteins cannot be eluted from the HaloLink™ Resin, which allows extensive washing to remove nonspecifically bound proteins without the danger of eluting HaloTag® fusion proteins. In addition, rapid binding, high binding capacity and low nonspecific binding contribute to reliable results.

Detection and Analysis of Protein:Protein Interactions

The covalent and oriented attachment of fusion proteins to HaloLink™ Resin provides an excellent choice to detect protein interactions using the pull-down method. HaloLink™ Resin is predominantly intended for detection of protein interactions when both protein partners are expressed in in vitro expression systems or for isolation of protein complexes from mammalian cells in vivo.

The typical pull-down assay comprises the protein of interest fused to a tag and a solid support that binds this protein. The fusion protein (also referred to as bait) bound to the resin is introduced to a pool of proteins containing potential binding partners (prey). The bait-prey complex is isolated from a complex protein mixture by resin precipitation and extensive washing to remove nonspecifically bound proteins. The specific binding partner is eluted, and the identity of the prey is usually determined by SDS-polyacrylamide gel electrophoresis or mass spectrometry. Pull-down assays are prone to false positives due to nonspecific binding of proteins. Covalent binding of HaloTag® fusion proteins to HaloLink™ Resin allows extensive washing without the fear of dissociation of HaloTag® fusion proteins from the resin. Additionally, low nonspecific binding of HaloLink™ Resin reduces the incidence of false positives, resulting in increased specificity.

Due to the advantageous properties of HaloLink™ Resin (stable binding, high on-rate, high capacity and low nonspecific binding), protein:protein interactions have been successfully detected where both bait and prey are expressed using in vitro protein expression systems. Furthermore, we have circumvented the need to precharge resin with bait and have isolated bait-prey protein complexes that were preformed in vitro or in vivo.

4. Protocols

Materials to be Supplied by the User

(Buffer compositions are provided in Section 7.A.)

- binding buffer
- wash buffer
- SDS-polyacrylamide gels
- rotating or shaking platform (i.e., tube rotator from Scientific Equipment Products; or other mixing devices such as the IKA-SCHÜTTLER MTS2)
- bovine serum albumen (BSA; Blot-Qualified BSA, Cat.# W3841)
- TNT® T7 Quick Coupled Transcription/Translation System (Cat.# L1170 or equivalent; see Section 7.C)
- centrifuge **Note**: Speed in rpm can be calculated from the following formula:

  $$RCF = (1.12)(r)(rpm/1{,}000)^2$$

  where r = radius in millimeters measured from the center of spindle to bottom of rotor bucket. In a standard size microcentrifuge 800 × *g* corresponds to 3,000rpm (rpm = revolutions per minute).

4.A. Immobilization of HaloTag® Fusion Proteins on HaloLink™ Resin

The protocol below is optimized to bind proteins synthesized using in vitro expression systems. We used the TnT® T7 Quick Coupled Transcription/ Translation System (Cat.# L1170). Other in vitro expression systems can be used (see Section 7.C and references 1 and 2). This protocol is a guideline and should be optimized for specific HaloTag® fusion proteins. Volumes of protein samples and resin can be scaled up or down depending on specific needs.

This protocol also can be used to immobilize proteins expressed in vivo in mammalian cells. Immobilized HaloTag® fusion proteins can be evaluated for enzymatic activity or protein:protein interactions, or can be cleaved from the resin with TEV protease (ProTEV Plus, Cat.# V6101, V6102) for use in other downstream applications.

Phase 1. Synthesis of the HaloTag® Fusion Proteins

For in vitro synthesis protocols, refer to references 1 and 2. For proteins expressed in mammalian cells, we added 100µl of cytosolic fraction (from cells grown in a 10cm culture dish, resuspended in 1ml of 10mM HEPES [pH 7.0] and lysed) to 100µl of resuspended HaloLink™ Resin. General guidelines on the number of cells to use and cell lysis conditions can be found in reference 3.

Note: For proteins expressed in mammalian cells, we recommend Protease Inhibitor Cocktail, 50X (Cat.# G6521). Some protease inhibitor cocktails, especially those containing, 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), will reduce binding efficiency significantly.

Phase 2. Resin Equilibration

1. Mix HaloLink™ Resin by inverting to obtain a uniform suspension. Dispense 50µl of HaloLink™ Resin into a 1.5ml microcentrifuge tube, and centrifuge for 1 minute at 800 × *g*. Carefully remove and discard the supernatant, leaving the resin at the bottom of the tube.
2. Add 400µl of binding buffer (Section 7.A). Mix thoroughly by inverting the tube. Centrifuge for 2 minutes at 800 × *g*. Carefully remove and discard the supernatant, leaving the resin at the bottom of the tube. Repeat Steps 1 and 2 two additional times for a total of 3 washes.
3. After the final wash, resuspend the resin in 50–100µl of binding buffer.

   Note: The volume used to resuspend the resin can be adjusted.

Phase 3. Binding of HaloTag® Fusion Protein

1. Add 40–50µl of the in vitro transcription/translation reaction containing the HaloTag® fusion protein to the equilibrated resin.
2. Incubate with mixing on a tube rotator (or equivalent device) for 30–60 minutes at room temperature. Incubate at 4°C if proteins are unstable; at 4°C, longer incubation time may be required. Make certain that the resin does not settle to the bottom of the tube; settling will reduce binding efficiency.
3. Centrifuge for 2 minutes at 800 × *g*. Save supernatant for analysis if desired.

Phase 4. Washing

1. Add 1ml of wash buffer (Section 7.A), and mix thoroughly by inverting the tube several times. Centrifuge for 2 minutes at 800 × *g*. Discard the wash. Repeat two additional times.
2. Add 1ml of wash buffer, and mix thoroughly by inverting the tube several times. Incubate at room temperature for 5 minutes with mixing. Centrifuge for 2 minutes at 800 × *g*. Discard the wash, and repeat this step.
3. Resuspend the resin carrying covalently attached HaloTag® fusion protein in the desired volume of a buffer compatible with downstream applications.

4.B. Detection and Analysis of Protein:Protein Interactions by Prebinding HaloTag® Fusion Proteins

This protocol describes a pull-down assay in which the HaloTag® fusion protein is synthesized using an in vitro expression system, then immobilized onto HaloLink™ Resin to be used as bait. A protein mixture containing the binding partner (prey) then is added to the immobilized bait and allowed to bind. Following washes, the prey protein is isolated and identified by the detection method of choice.

This protocol is optimized for use with proteins synthesized in the TnT® T7 Quick Coupled Transcription/ Translation System (Cat.# L1170). Other in vitro expression systems can be used (see Section 7.C and references 1 and 2). The protocol also can be used to immobilize proteins expressed in vivo in mammalian cells. This protocol is a guideline and should be optimized for specific HaloTag® fusion proteins. Volumes of protein sample and resin can be scaled up or down depending on specific needs.

It is important to include the appropriate controls in the pull-down experiment to demonstrate that results are due to specific interactions. A negative control (resin only) without HaloTag® fusion bait protein should be set up in parallel with the experimental sample to assay nonspecific binding to the resin.

Phase 1. Synthesis of the HaloTag® Fusion Bait Protein

For in vitro synthesis protocols, refer to references 1 and 2. While synthesizing the bait protein, equilibrate the HaloLink™ Resin as described below.

4.B. Detection and Analysis of Protein:Protein Interactions by Prebinding HaloTag® Fusion Proteins (continued)

Phase 2. Resin Equilibration

1. Mix the HaloLink™ Resin by inverting to obtain a uniform suspension.
2. For each pull-down experiment, dispense 50µl of HaloLink™ Resin suspension into two 1.5ml microcentrifuge tubes (experimental sample and negative control). Centrifuge for 1 minute at 800 × *g*. Carefully remove and discard the supernatant, leaving the resin at the bottom of the tube.
3. Add 400µl of binding buffer (Section 7.A). Mix thoroughly by inverting the tube. Centrifuge for 2 minutes at 800 *x g*. Carefully remove and discard the supernatant, leaving the resin at the bottom of the tube. Repeat twice for a total of 3 washes.
4. After the final wash, resuspend the resin in 50–100µl of binding buffer.

   Note: The volume used to resuspend the resin can be adjusted.
5. Add cofactors, detergents or other reagents needed for specific protein-protein interactions.

Phase 3. Binding of HaloTag® Fusion Bait Protein

1. To the experimental sample, add 40–50µl of the in vitro transcription/translation reaction containing the HaloTag® fusion protein.
2. To the negative control (resin without bait), add an equal volume of binding buffer or TnT® T7 Quick Coupled Transcription/Translation reaction without DNA template.
3. Incubate with mixing on a tube rotator (or equivalent device) for 30–60 minutes at room temperature. Incubate at 4°C if proteins are unstable; at 4°C, longer incubation time may be required. Make certain that the resin does not settle to the bottom of the tube; settling will reduce binding efficiency.

   Note: During this incubation, prey can be synthesized as described below (Phase 5, Step 1).
4. At the end of the bait-binding incubation, centrifuge for 2 minutes at 800 × *g*. Save supernatant for analysis if desired.

Phase 4. Washing

1. Add 1ml of wash buffer (Section 7.A), and mix thoroughly by inverting the tube several times. Centrifuge for 2 minutes at 800 × *g*. Discard the wash. Repeat two additional times.
2. Add 1ml of wash buffer, and mix thoroughly by inverting the tube several times. Incubate at room temperature for 5 minutes with mixing. Centrifuge for 2 minutes at 800 × *g*. Discard the wash, and repeat this step.
3. Resuspend the resin carrying covalently attached HaloTag® fusion protein in 100µl of wash buffer. Store the resin with immobilized bait at 4°C until prey synthesis is finished if necessary.

Phase 5. Synthesis and Capture of Prey Protein

1. For in vitro synthesis refer to references 1 and 2. To detect the prey protein later during analysis, label the protein with [^{35}S] methionine or FluoroTect™ GreenLys in vitro Translation Labeling System (Cat.# L5001). Alternatively proteins can be detected by Western analysis.
2. To each of the experimental and negative control samples, add 20µl of the prey protein reaction, and incubate with mixing on a tube rotator (or equivalent device) for 1–2 hours at room temperature. Incubate at 4°C if proteins are unstable; at 4°C, longer incubation time may be required. Make certain that the resin does not settle to the bottom of the tube; settling will reduce binding efficiency.
3. At the end of the prey-capture incubation, centrifuge for 2 minutes at 800 × *g*. Discard the supernatant.

Phase 6. Washing

Note: Stability of different protein-protein interactions is protein pair-specific and depends on the affinity of the interaction. Wash conditions might need to be optimized.

1. Add 1ml of wash buffer (Section 7.A), and mix thoroughly by inverting the tube several times. Centrifuge for 2 minutes at 800 × *g*. Discard the wash. Repeat two additional times.
2. Add 1ml of wash buffer, and mix thoroughly by inverting the tube several times. Incubate at room temperature for 5 minutes with mixing. Centrifuge for 2 minutes at 800 × *g*. Discard the wash, and repeat this step.

Phase 7. Elution

1. Add 20µl of 1X SDS loading buffer (Section 7.A).
2. Incubate at 90°C for 2–5 minutes, remove supernatant and load on an SDS-PAGE gel for analysis using the detection method chosen.

4.C. Detection and Analysis of Protein:Protein Interactions by Isolation of Preformed Bait-Prey Complexes

This protocol describes a pull-down assay in which both the HaloTag® fusion protein bait and prey protein are synthesized in vitro, mixed and allowed to form complexes. Bait-prey complexes then are isolated with the HaloLink™ Resin, and prey protein is identified.

This protocol is optimized for use with proteins synthesized in the TnT® T7 Quick Coupled Transcription/ Translation System (Cat.# L1170). Other in vitro expression systems can be used (see Section 7.C and references 1 and 2). This protocol is a guideline and should be optimized for specific HaloTag® fusion proteins. Volumes of protein samples and resin can be scaled up or down, depending on specific needs.

Be sure to include the appropriate controls in the pull-down experiment to demonstrate that results are due to specific interactions. A negative control (resin only) without HaloTag® fusion bait protein should be set up in parallel with the experimental sample to assay nonspecific binding to the resin.

4.C. Detection and Analysis of Protein:Protein Interactions by Isolation of Preformed Bait-Prey Complexes (continued)

Phase 1. Synthesis of the HaloTag® Fusion Bait Protein and Prey Protein

Synthesize both the HaloTag® fusion bait and prey proteins concurrently. For in vitro synthesis protocols, refer to references 1 and 2.

To detect prey protein during analysis, label the prey protein during synthesis with [^{35}S]methionine or FluoroTect™ GreenLys in vitro Translation Labeling System (Cat.# L5001). Alternatively the prey protein can be detected by Western analysis.

Phase 2. Bait and Prey Proteins Complex Formation

1. For the experimental sample, combine 20µl of bait in vitro synthesis reaction with 20µl of prey in vitro synthesis reaction.
2. For the negative control (no bait), combine 20µl of the prey in vitro synthesis reaction with 20µl of binding buffer or TnT® T7 Quick Coupled Transcription/Translation reaction without DNA template.
3. Add cofactors, detergents or other reagents needed for interaction of the specific protein pair, and incubate at room temperature for 1 hour with rotation. The temperature and time of this incubation might need to be optimized for different protein pairs.

   Note: During this incubation, equilibrate the HaloLink™ Resin as described below.

Phase 3. Resin Equilibration

1. Mix HaloLink™ Resin by inverting to obtain a uniform suspension. For each pull-down experiment dispense 50µl of HaloLink™ Resin into two 1.5ml microcentrifuge tubes (experimental sample and negative control). Centrifuge for 1 minute at 800 × *g*. Carefully remove and discard the supernatant, leaving the resin at the bottom of the tube.
2. Add 400µl of binding buffer (Section 7.A). Mix thoroughly by inverting the tube. Centrifuge for 2 minutes at 800 × *g*. Carefully remove and discard the supernatant, leaving the resin at the bottom of the tube. Repeat Steps 1 and 2 two additional times for a total of 3 washes.
3. After the final wash, resuspend the resin in 50–100µl of binding buffer.
4. Add cofactors, detergents or other reagents needed for interaction of the specific protein pair.

Phase 4. Capture of Bait-Prey Complexes

1. To the resin, add 20–40µl of the appropriate mixtures from Phase 2 (experimental sample and negative control). Use 20µl as a starting point, and increase the volume if protein expression level is low.
2. Incubate with mixing on a tube rotator (or equivalent device) for 1–2 hours at room temperature. Incubate at 4°C if proteins are unstable; at 4°C, longer incubation time may be required. Make certain that the resin does not settle to the bottom of the tube; settling will reduce binding efficiency.
3. Centrifuge for 2 minutes at 800 × *g*. Save supernatant for analysis if desired.

Phase 5. Washing

Note: Stability of different protein:protein interactions is protein pair-specific and depends on the affinity of the interaction. Wash conditions might need to be optimized.

1. Add 1ml of wash buffer (Section 7.A), and mix thoroughly by inverting the tube several times. Centrifuge for 2 minutes at 800 × *g*. Discard the wash. Repeat two additional times.
2. Add 1ml of wash buffer, and mix thoroughly by inverting the tube several times. Incubate at room temperature for 5 minutes with mixing. Centrifuge for 2 minutes at 800 × *g*. Discard the wash, and repeat this step.

Phase 6. Elution

1. Add 20µl of 1X SDS loading buffer (Section 7.A).
2. Incubate at 90°C for 2–5 minutes, remove supernatant and load on an SDS-PAGE gel for analysis using the detection method chosen.

5. General Considerations

5.A. Creating HaloTag® Fusion Protein Constructs

Instructions to create HaloTag® Fusion Protein constructs can be found in the *Flexi® Vector Systems Technical Manual* #TM254 (4) and *HaloTag® Technology: Focus on Imaging Technical Manual* #TM260 (3).

We suggest that protein synthesis be verified. Section 5.C provides guidelines to detect HaloTag® fusion proteins with a fluorescent TMR-conjugated ligand. If nontagged proteins are synthesized to detect protein:protein interactions, we suggest using [^{35}S]methionine or FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System (Cat.# L5001) for detection.

5.B. Performing the TnT® T7 Quick Coupled Transcription/Translation Reaction

We recommend using the TnT® T7 Quick Coupled Transcription/Translation System for protein expression. Purified plasmid DNA used for the transcription/translation reaction should be free of residual ethanol and salts. To isolate clean DNA, we recommend using the Wizard® Plus Minipreps DNA Purification System, Wizard® Plus SV Minipreps DNA Purification System or PureYield™ Plasmid Miniprep System (Cat.# A7100, A1330 and A1221, respectively). DNA prepared by the standard alkaline lysis method described by Sambrook, Fritsch and Maniatis (5) also is sufficiently clean for use in the TnT® T7 Quick Coupled Transcription/Translation System reaction.

For most plasmid constructs, optimal results are obtained when 1µg of plasmid DNA template is used. However, we have used 0.5–2.0µg of DNA template and obtained satisfactory levels of translation. The use of more than 1µg of plasmid does not necessarily increase the amount of protein produced. Linearized templates produced by restriction enzyme digestion of plasmids should be purified before use in an in vitro transcription/translation reaction. For more information, refer to the *TnT® Quick Coupled Transcription/Translation System Technical Manual* #TM045, and Hurst, R. *et al.* (1).

5.C. Detecting HaloTag® Fusion Proteins

The HaloTag® technology offers a quick and convenient way to test protein expression by fluorescent detection using the HaloTag® TMR Ligand (Cat.# G8251, G8252; Figure 1). The fluorescent TMR-conjugated ligand can be added to a fraction of the in vitro transcription/translation reaction containing HaloTag® fusion protein or to a fraction of cell lysate and analyzed by SDS gel electrophoresis (3). The same protocol can be used to monitor binding of HaloTag® fusion proteins to the HaloLink™ Resin by analyzing the starting material and supernatant recovered after protein binding. This supernatant contains unbound protein.

1. Prepare a 500-fold dilution of HaloTag® TMR Ligand stock solution (5mM) in binding buffer (or 1X TBS buffer) for a final concentration of 10µM.
2. Mix 2µl of in vitro transcription/translation reaction containing the HaloTag® fusion protein or an equivalent amount of unbound fraction with 1µl of 10µM HaloTag® TMR Ligand.
3. Add binding buffer or 1X TBS buffer to a final volume of 10µl. Incubate at room temperature, protected from light, for 30 minutes.
4. Remove 5µl of the reaction, add 5µl of 2X SDS gel loading buffer and heat at 70°C for 5 minutes. Separate on an SDS-polyacrylamide gel. Analyze on a fluorescent detection scanner (e.g., Typhoon®, GE Healthcare Bio-sciences).

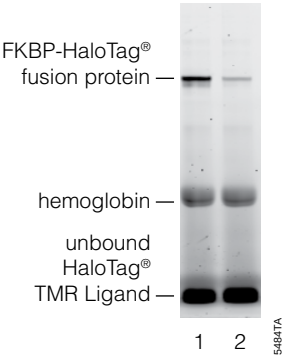

Figure 1. Detection of HaloTag® fusion proteins using HaloTag® TMR Ligand. Lane 1. TnT® T7 Quick Coupled Transcription/Translation System reaction expressing HaloTag® FK506-binding protein (FKBP) fusion protein. **Lane 2.** FKBP-HaloTag® fusion protein present in the unbound fraction. Five microliters of the HaloTag® TMR Ligand labeling reaction was loaded onto the gel for both lanes. Hemoglobin is present in the rabbit reticulocyte lysate preparations; it autofluoresces and thus appears in both gel lanes.

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

Symptoms	Causes and Comments
Inefficient immobilization of HaloTag® fusion protein to resin	Increase the volume of the cell-free translation reaction to the HaloLink™ Resin.
	Increase the volume of the in vitro transcription/translation reaction added to the HaloLink™ Resin containing the bait, prey or bait-prey mix.
	Adjust the volume of the HaloLink™ Resin used.
	Increase the time allowed to bind the HaloTag® fusion protein to the HaloLink™ Resin.
	Adjust the binding temperature.
	Proteolysis of translation product. Add protease-inhibitor cocktail. We recommend BaculoGold® protease inhibitor cocktail (BD Biosciences Cat.# 554779). Some protease inhibitor cocktails, especially those containing 4-(2-amino ethyl) benzenesulfonyl fluoride hydrochlorid (AEBSF) will reduce binding efficiency.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
High Background	Volume of sample containing HaloTag® protein was too large. Reduce the volume of the sample added to the HaloLink™ Resin.
	Nonspecific interactions can be diminished by increasing wash stringency. Increase the number and/or volume of washes and include salt or detergents in the wash buffer.
	Increase the amount of BSA in the wash bffer. Use Blot-Qualified BSA (Cat. #W3841).
	Add IGEPAL® CA-630 at 0.05% final concentration or 0.5% Triton®X-100 or 5% glycerol to reduce background.
	Too much HaloLink™ Resin resulted in high background. Reduce the amount of resin added to the sample.
HaloTag® fusion protein not detected with HaloTag® TMR Ligand	Too much sample was loaded onto the SDS polyacrylamide gel. Reduce the volume of the in vitro transcription/ translation reaction loaded (e.g. 1–5µl). Too much sample causes hemoglobin precipitation and prevents protein migration into the gel due to aggregation.
	Fluorescent scanner does not have appropriate filters and/or sensitivity. Use a fluorescent scanner with the appropriate sensitivity (e.g., Typhoon®, GE Healthcare Biosciences). For scanners with lower sensitivity, sample volume may need to be increased, but use caution due to hemoglobin precipitation (see comment above).
	Samples may be overheated. Change the denaturation conditions to 60°C for 5–10 minutes.

7. Appendix

7.A. Composition of Buffers

wash buffer

100mM	Tris (pH 7.6)
150mM	NaCl
1mg/ml	BSA
0.05%	IGEPAL® CA-630 (Sigma Cat# I3021 or I8896)

Note: If IGEPAL® CA-630 interferes with the activity of the protein of interest, IGEPAL® CA-630 can be eliminated or reduced to 0.001%. However, this may result in higher nonspecific binding. If IGEPAL® CA-630 is reduced in concentration or eliminated, we recommend the use of 0.5% Triton® X-100 or 5% glycerol. BSA may also be eliminated if it interferes with protein activity, but higher nonspecific binding may result. Buffers other than Tris can be used in this protocol.

1X TBS buffer

100mM	Tris (pH 7.6)
150mM	NaCl

binding buffer

100mM	Tris (pH 7.6)
150mM	NaCl
0.05%	IGEPAL® CA-630 (Sigma Cat# I3021 or I8896)

Note: IGEPAL® CA-630 is added to prevent the resin from sticking to the sides of the tube. (IGEPAL® is an NP40 analog.) The effective range of IGEPAL® concentration is 0.001–0.05%. For ease of use, we recommend preparing a 10% stock solution of IGEPAL® and making further dilutions from this solution.

Important: Buffers containing IGEPAL® should be stored at room temperature and used within one week of preparation.

4X SDS gel loading buffer

0.24M	Tris-HCl (pH 6.8)
3mM	bromophenol blue
50.4%	glycerol
0.4M	dithiothreitol
2%	SDS

7.B. References

1. *TnT® Quick Coupled Transcription/Translation Systems Technical Manual*, #TM045, Promega Corporation.
2. *TnT® Coupled Reticulocyte Lysate Systems Technical Bulletin*, #TB126, Promega Corporation.
3. *HaloTag® Technology: Focus on Imaging Technical Manual*, #TM260, Promega Corporation.
4. *Flexi® Vector Systems Technical Manual*, #TM254, Promega Corporation.
5. Sambrook, J. *et al.* (1989) *Molecular Cloning: A Laboratory Manual, Second Edition*. Cold Spring Harbor Press, Cold Spring Harbor, NY.

7.C. Related Products

Product	Size	Cat.#
HaloTag® Protein Purification System	25ml	G6280
HaloTag® Protein Purification System Sample Pack	2.5ml	G6270
HaloLink™ Magnetic Beads	40 reactions	G9311
Blot-Qualified BSA	10g	W3841
ProTEV Plus	1,000u	V6101
	8,000u	V6102

HaloTag® Product for Protein:DNA Interactions

Product	Size	Cat.#
HaloCHIP™ System	20 reactions	G9410

Protein Array Systems

Product	Size	Cat.#
HaloLink™ Array (TnT® T7 Quick) Two-Slide System	two 50-well arrays	G6140
HaloLink™ Array (TnT® SP6 Wheat Germ) Two-Slide System	two 50-well arrays	G6180
HaloLink™ Array Six-Slide System	6 slides	G6190

Detection System

Product	Size	Cat.#
FluoroTect™ GreenLys in vitro Translation Labeling System	40 reactions	L5001

For Laboratory Use.

HaloTag® Flexi® Vectors

Product	Size	Cat.#
pFN18A HaloTag® T7 Flexi® Vector	20µg	G2751
pFN18K HaloTag® T7 Flexi® Vector	20µg	G2681
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
pFC14A HaloTag® CMV Flexi® Vector	20µg	G9651
pFC14K HaloTag® CMV Flexi® Vector	20µg	G9661
pFC15A HaloTag® CMVd1 Flexi® Vector	20µg	G1611
pFC15K HaloTag® CMVd1 Flexi® Vector	20µg	G1601
pFC16A HaloTag® CMVd2 Flexi® Vector	20µg	G1591
pFC16K HaloTag® CMVd2 Flexi® Vector	20µg	G1571
pFC17A HaloTag® CMVd3 Flexi® Vector	20µg	G1551
pFC17K HaloTag® CMVd3 Flexi® Vector	20µg	G1321
pFN21A HaloTag® CMV Flexi® Vector	20µg	G2821
pFN21K HaloTag® CMV Flexi® Vector	20µg	G2831
pFN22A HaloTag® CMVd1 Flexi® Vector	20µg	G2841
pFN22K HaloTag® CMVd1 Flexi® Vector	20µg	G2851
pFN23A HaloTag® CMVd2 Flexi® Vector	20µg	G2861
pFN23K HaloTag® CMVd2 Flexi® Vector	20µg	G2871
pFN24A HaloTag® CMVd3 Flexi® Vector	20µg	G2881
pFN24K HaloTag® CMVd3 Flexi® Vector	20µg	G2981
HaloTag® Flexi® Vectors—CMV Deletion Series Sample Pack	9 × 2µg	G3780

7.C. Related Products (continued)

HaloTag® Fluorescent Ligands

Product	Size	Cat.#
HaloTag® TMR Ligand	30µl	G8251
HaloTag® diAcFAM Ligand	30µl	G8272
HaloTag® Coumarin Ligand	30µl	G8581
HaloTag® Oregon Green® Ligand	30µl	G2801
HaloTag® Alexa Fluor® 488 Ligand	30µl	G1001

Available in additional sizes.

Flexi® Cloning Systems

Product	Size	Cat.#
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

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 GermProtein Expression System	4 × 300µl	L3260
	1 × 300µl	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

8. Summary of Changes

The following change was made to the 9/16 revision of this document:

1. Notes referencing Wheat Germ Extract Plus (discontinued) were removed from pages 4, 5 and 8.