Technical Manual

# HaloCHIP™ System

INSTRUCTIONS FOR USE OF PRODUCT G9410.

www.promega.com

PRINTED IN USA.
Revised 12/15

Part# TM075

Promega

# HaloCHIP™ System

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



## 1. Description

The HaloCHIP™ System[a,b] is a novel system that covalently captures crosslinked protein:DNA complexes from mammalian cells without the need for antibodies, providing a robust and efficient alternative to the standard chromatin immunoprecipitation (ChIP) method (1; Figure 1). The HaloCHIP™ System takes advantage of the HaloTag® protein (2,3), which can be fused to any protein and mediates a covalent interaction with various ligands, including a resin-based ligand, HaloLink™ Resin[a,c]. For this application, a DNA-binding protein of interest is fused to the HaloTag® protein via cloning in a HaloTag® vector. The recombinant vector construct is transfected into cells for either stable or transient expression. Then the cells are treated with formaldehyde to induce covalent protein:DNA crosslinks, lysed and sonicated to shear the chromatin into smaller fragments for purification by the HaloCHIP™ System. Using the HaloCHIP™ System, these crosslinked complexes can be directly captured from the lysate through covalent binding between the HaloTag® moiety of the fusion protein and the HaloLink™ Resin. Stringent washing removes nonspecific proteins and DNA.


**Promega Corporation** · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA
Toll Free in USA 800-356-9526 · Phone 608-274-4330 · Fax 608-277-2516 · **www.promega.com**
Printed in USA. Part# TM075
Revised 12/15

Subsequent heating of the HaloTag® fusion protein:DNA complexes on the resin reverses the crosslinks between the DNA and the fusion protein and releases the captured DNA fragments, which can be subsequently purified using PCR product purification kits. Analysis of the DNA fragments can be performed using a variety of methods including PCR and microarray analysis. There are two possible controls recommended for the HaloCHIP™ experiments and either can be used to provide good estimates of background DNA. The first control is to process untransfected cells throughout the HaloCHIP™ array in parallel with the experimental sample (Figure 1). The second control, termed the Blocking Ligand Control, is established by splitting the lysate equally into two tubes prior to capture on the the HaloLink™ Resin; one tube is labeled as experimental sample and one as control sample. The control sample is incubated with a blocking ligand, which binds to the HaloTag® protein and prevents interaction with the HaloLink™ Resin (Figure 1).

## 2. Benefits of the HaloCHIP™ System

One of the challenges of the traditional ChIP method is the availability of

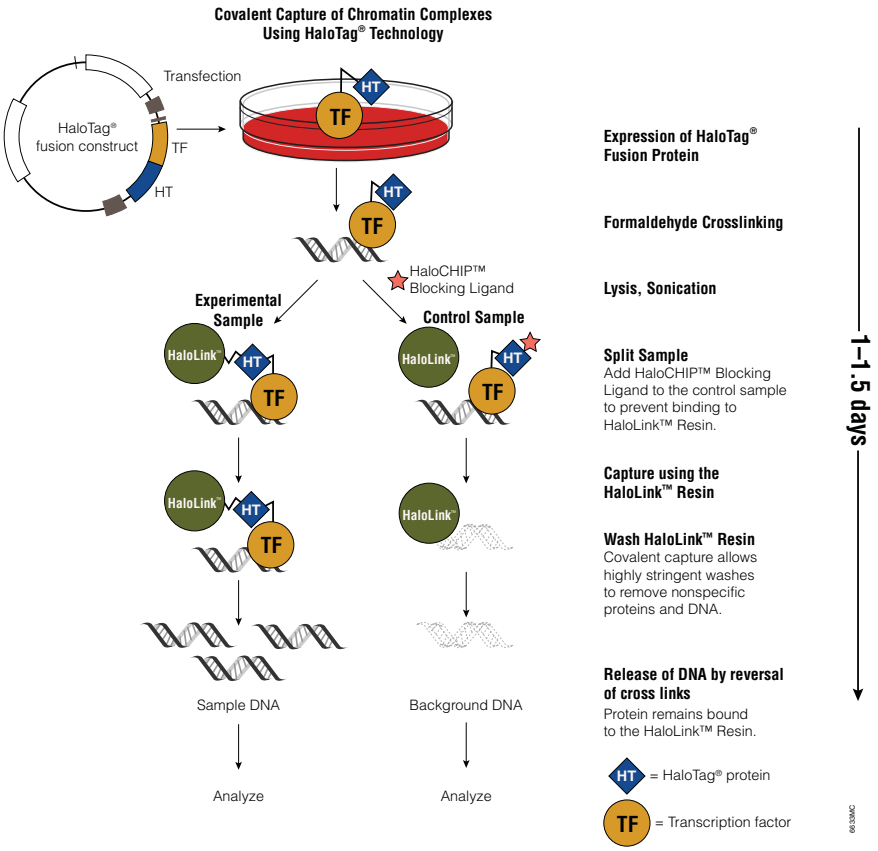


**Figure 1. Schematic diagram of the HaloCHIP™ System.**

highly specific antibodies certified to recognize crosslinked epitopes. In the HaloCHIP™ method, recombinant HaloTag® fusion proteins crosslinked to DNA can be directly captured on the HaloLink™ Resin, thus eliminating the need for antibodies. Another important benefit of this method is the covalent binding of the HaloTag® fusion protein to the HaloLink™ Resin, resulting in a complete covalent linkage between the resin, protein and DNA. This allows extensive, stringent washing that is not possible with noncovalent interactions and results in reduced background and an increased signal-to-noise ratio. Unlike other affinity tags, the HaloTag® protein also allows the capture of complexes in very low abundance and concentration within the cell because the covalent bond formed between the HaloTag® protein and the HaloLink™ Resin does not disassociate. The rapid binding of the HaloTag® fusion protein to the HaloLink™ Resin can be completed within hours, resulting in a much shorter and easier protocol. Sample recovery is highly efficient, allowing smaller sample sizes to be used for each experiment. Using smaller sample sizes results in a lower overall background.

## 3. Product Components and Storage Conditions

| Product | Size | Cat.# |
|---|---|---|
| HaloCHIP™ System | 20 reactions | G9410 |

Cat.# G9410 includes G9411 and G5491. Each system contains sufficient reagents for 20 reactions. **This system does not contain IGEPAL® CA-630, which is required to prepare the Resin Equilibration Buffer (Section 4).**

Cat.# G9411 includes:

- 0.5ml HaloLink™ Resin
- 40ml Mammalian Lysis Buffer
- 25ml High Salt Wash Buffer
- 100ml TE Buffer, 1X
- 8ml Reversal Buffer
- 150ml Nuclease-Free Water

Cat.# G5491 includes:

- 30µl HaloCHIP™ Blocking Ligand

**Storage Conditions:** The TE Buffer, 1X, Reversal Buffer and Nuclease-Free Water may be stored at room temperature. Store the HaloLink™ Resin, Mammalian Lysis Buffer and High Salt Wash Buffer at 4°C. Store the HaloCHIP™ Blocking Ligand at –20°C.

## 4. Materials and Preparation

**Materials to Be Supplied by the User**

- cells for transfection or appropriate stable transformed cell line expressing HaloTag® fusion protein construct
- medium required for growing cells
- transfection reagents
- cloning vectors encoding the HaloTag® protein (See Section 9, Related Products, for a list of vectors.)
- PBS, tissue culture certified
- BSA (e.g., Cat.# R3961; 10mg/ml; optional)
- formaldehyde, A.C.S. reagent (e.g., Sigma Cat.# 252549)
- 1.25M glycine (pH 7.0; this is a 10X solution)
- IGEPAL® CA-630 (Sigma Cat.# I8896)
  **Note:** IGEPAL® CA-630 is chemically indistinguishable from Nonidet® P-40.
- cell scraper
- sonicator
  **Note:** This protocol is optimized for the Misonix Sonicator 3000 using the Microtip Probe 418 or cuphorn; see protocol for details. If using a different sonicator, we recommend using a low-output sonicator with a microtip or cuphorn.
- centrifuge
  **Note:** Speed in rpm can be calculated from the following formula: $RCF = (1.12)(r)(rpm/1000)^2$ where r = radius in millimeters measured from center of spindle to bottom of rotor bucket. In a standard microcentrifuge, 800 × *g* corresponds to 3,000rpm (rpm = revolutions per minute).
- heating block (e.g., ThermoKool from Barnstead/Thermolyne or other heating devices capable of stably maintaining temperature at 65°C)
- glass dounce homogenizer (0.5–2ml) or 25- or 27-gauge needle and a 1ml syringe
- tube rotator (e.g., Barnstead International model # 400110, Scientific Equipment Products Cat.# 60448 or equivalent device)
- SDS-PAGE gels
- 1–2% agarose gels
- PCR product purification system
- protease inhibitor cocktail (optional)
  **Note:** If adding a protease inhibitor cocktail is desired, we recommend BaculoGold™ Protease Inhibitor Cocktail (BD Biosciences, Cat.# 554779). Avoid cocktails of unknown composition or those known to contain AEBSF (4-(2-aminoethyl benzenesulfonyl fluoride hydrocholoride). AEBSF will significantly reduce binding efficiency.

**Before Starting**

1. **Prepare HaloTag® Fusion Protein:** Clone the desired DNA binding protein coding sequence into a HaloTag® vector according to manufacturer's recommendation.

2. **Prepare Cells:** The following protocol has been optimized for a single HaloCHIP™ reaction. We recommend using 1–2 wells (9.4cm$^2$/well) of a standard six-well plate, and plate the cells at a density of 4–8 × $10^5$ cells/well. For either the untransfected or blocking ligand control, prepare the same number of cells. For transient transfections, grow cells to 70–80% confluency, and transfect according to the manufacturer's recommendations. Perform formaldehyde crosslinking (Phase 2, Step 1) at approximately 24 hours post-transfection. For adherent stable cell lines or the untransfected control, plate the cells similarly, then crosslink after reaching 80–90% confluency.

3. **Prepare HaloLink™ Resin Equilibration Buffer:** Add IGEPAL® CA-630 to TE Buffer (pH 8.0) to a final concentration of 0.1%.

**Note:** Only prepare Resin Equilibration Buffer sufficient for a given set of experiments; do **not** add IGEPAL® CA-630 to the entire amount of TE Buffer, 1X, supplied in the kit.

Due to the solubility properties of IGEPAL® CA-630, we recommend that you first prepare a 10% stock solution of IGEPAL® CA-630 (in water) and use this solution to prepare the HaloLink™ Resin Equilibration Buffer. The effective range of IGEPAL® CA-630 in the HaloCHIP™ System is 0.01–0.1%.

**Note:** Store buffers containing IGEPAL® CA-630 at room temperature and use within one week of preparation.

4. **Optimize Sonication Conditions:** Sonication conditions can be optimized using transfected or untransfected cells. The protocols included were optimized for the Misonix Sonicator 3000 with a Microtip Probe 418 or cuphorn (Section 5.A, Phase 2, Step 15). If you are using a different sonicator, we strongly recommend following the HaloCHIP™ protocol up to the point of sonication (Section 5.A, Phase 2, Step 15). At this point, you will need to evaluate the sonicated DNA fragment size (Section 5.C) before continuing with the entire protocol. After you have optimized the sonication conditions, use transfected cells to determine the efficiency of HaloTag® binding (Section 5.D) to ensure that the sonication conditions are optimal for HaloLink™ Resin capture.

## 5. Protocols

This protocol has been optimized for mammalian cells. If you are using other cell types, the crosslinking, lysis and sonication steps will need to be optimized. Do **not** use SDS, IGEPAL® CA-630, NP-40 or Tween®-20 detergents in the lysis buffer (Phase 2, Step 13), as these detergents inhibit capture with the HaloLink™ Resin.

### 5.A. HaloCHIP™ System Protocol

The following protocol is designed for a single HaloCHIP™ experiment. If using the untransfected control, follow protocol as recommended for the HaloCHIP™ experimental sample. If using the blocking ligand control, follow protocol as recommended to through the end of Phase 2 to the start of Phase 3. Afterward, follow recommendations for preparation of blocking ligand control included in Phase 3, Steps 1–3. Prepare one tube of HaloLink™ Resin for each HaloCHIP™ experimental and control reactions (Phase 1, Step 1).

**Phase 1. Resin Equilibration**

1. Mix the HaloLink™ Resin by inverting the tube until a uniform suspension is obtained. Dispense 75µl of HaloLink™ Resin into a single 1.5ml microcentrifuge tube for each HaloCHIP™ reaction sample (e.g., if performing an experimental HaloCHIP™ sample along with both blocking ligand and untransfected controls, prepare three tubes of HaloLink™ Resin).
2. Centrifuge at 800 × *g* for 2 minutes. Carefully remove and discard the supernatant, leaving the HaloLink™ Resin at the bottom of the tube.
3. Add 400µl of Resin Equilibration Buffer (as prepared in Section 4) to each tube of resin. Mix thoroughly by inverting the tube several times. Centrifuge for 2 minutes at 800 × *g*. Carefully remove and discard the supernatant, leaving the HaloLink™ Resin at the bottom of the tube.
4. Repeat Step 3 two additional times for a total of three washes. After the third wash, **do not** remove the Resin Equilibration Buffer until you are ready to use the HaloLink™ Resin in Phase 3, Step 2. This will prevent the HaloLink™ Resin from drying out. Store the prepared HaloLink™ Resin tubes at 4°C for up to 24 hours.

**Phase 2. Crosslinking and Sonication**

The following protocol is designed for 4–8 × $10^5$ adherent cells plated in a standard six-well plate (pretransfection). For scale-up of the HaloCHIP™ experiment, refer to Section 5.B for protocol guidelines. Crosslinking is performed approximately 24 hours post-transfection. We recommend performing Steps 1–9 in a tissue culture hood.

1. Prewarm an amount of the medium (equivalent to the volume of the culture) to room temperature. Add formaldehyde (37% stock) to a final concentration of 0.75–1% and mix well.

   **Optional:** Tissue-culture-certified PBS may be used in the place of medium.

   **Note:** Formaldehyde is easily oxidized, reducing its effectiveness in crosslinking. Replace the formaldehyde with fresh every 3 months.

   **Note:** Formaldehyde is toxic by inhalation or ingestion and is readily adsorbed through the skin. Take suitable precautions when using and disposing of formaldehyde solutions.

2. Aspirate the old medium from the cells, and gently add an equivalent amount of formaldehyde-containing medium or formaldehyde-containing PBS. Swirl the plate gently.

3. Incubate for 10 minutes at room temperature.

4. Slowly, and with constant swirling of the plate containing the cells, add 1.25M glycine (pH 7.0) to a final concentration of 125mM to quench the crosslinking. For 2ml of medium or PBS, add 220µl of glycine. A uniform yellow color will appear.

5. Incubate for 5 minutes at room temperature.

6. Aspirate the medium, add 2ml of ice-cold PBS and gently swirl the plate.

7. Aspirate the PBS, add 2ml of ice-cold PBS and gently swirl the plate.

8. Aspirate the PBS, add 0.75–2ml of ice-cold PBS to each well, and collect cells by scraping using a cell scraper or rubber policeman.

   **Note:** A larger volume or adding a PBS wash step can improve cell recovery.

9. Pool the cells recovered from each well into a single, prechilled 1.5ml microcentrifuge tube (1–2 wells) or a 15ml conical tube, and immediately place the tube on ice.

10. Centrifuge at 2,000 × *g* for 5 minutes at 4°C.

11. Carefully remove the supernatant.

12. Freeze cell pellets at –70°C for 10 minutes, then thaw at room temperature.

    **Note:** At this point, cells can be stored at –70°C and processed later.

    **Optional:** If desired, a cytoplasmic lysis can be performed to reduce cytoplasmic contribution by following the protocol in Section 5.E.

**5.A. HaloCHIP™ System Protocol (continued)**

**Phase 2. Crosslinking and Sonication (continued)**

13. Resuspend the cell pellet from the cells harvested from up to six wells in 650µl of cold Mammalian Lysis Buffer, vortex briefly and incubate on ice for 15 minutes.

**Notes**

1. If adding a protease inhibitor cocktail to the lysis buffer is desired, we recommend BaculoGold™ Protease Inhibitor Cocktail (BD Biosciences, Cat.# 554779). Avoid cocktails of unknown composition or those known to contain AEBSF (4-(2-aminoethyl benzenesulfonyl fluoride hydrocholoride). AEBSF will significantly reduce binding efficiency to the HaloLink™ Resin.

2. If you are only using one well or scaling up to six wells, do not change the volume of Mammalian Lysis Buffer used. Different volumes will affect the sonication. If processing more than six wells, perform separate reactions and pool the purified DNA.

14. Lyse cells using mechanical disruption (e.g., use a 2ml glass dounce homogenizer, 25–30 strokes on ice, or pass the cells 4–6 times through a 25- or 27-gauge needle).

15. Sonicate the cell lysate in an ice-water bath to obtain DNA fragments 500–1,500bp in size. The recommendations below are for the Misonix Sonicator 3000 MicroTip Probe 418 and cuphorn. If you are optimizing the sonication conditions, continue to Section 5.C.

**Recommended Sonication Conditions for Misonix Sonicator 3000:**

If you are using a sonicator other than the Misonix Sonicator 3000, you will need to determine the optimal sonication conditions (Section 5.C).

Using the Misonix Sonicator 3000 with the Microtip Probe 418, place the 650µl of lysate prepared above in an ice bath. Submerge the Microtip into the lysate near the bottom of the tube. Set the output to 2.5 and perform 6 cycles of alternating 10 seconds on and 10 seconds off, followed by 2 minutes on ice. Repeat an additional round of 6 cycles of alternating 10 seconds on and 10 seconds off. The total sonication time will be 120 seconds.

If you are using the Misonix Sonicator 3000 cuphorn, pre-cool the cuphorn using an ice-water bath for 10 minutes using a volume of approximately 150ml. Place tubes in the tube holder so they are in contact with the cuphorn. Set the output to 4.0 and perform 15 cycles of alternating 10 seconds on and 15 seconds off.

**Note:** Excessive sonication and heating of the sample during sonication will denature the HaloTag® protein, resulting in decreased binding to the HaloLink™ Resin.

**Phase 3. Capture and Release of DNA**

1. Centrifuge the sonicated samples at 14,000 × *g* for 5 minutes at 4°C.

   **Note:** If using the blocking ligand control, pool supernatants after centrifugation. Then divide the pooled sample into two equal samples of 600µl each. Label one sample as the experimental sample and the other as the blocked ligand control sample. To the control sample only, add 0.6µl of the HaloCHIP™ Blocking Ligand directly to the lysate for a final concentration of 5.0µM (see Section 7). Incubate both samples for 30 minutes at room temperature with constant mixing before proceeding to Step 2.

   **Optional:** Save the remaining supernatant (input material) to analyze the efficiency of HaloTag® fusion protein binding (Section 5.D).

   **Note:** Due to the fluorescent dye attached to the HaloCHIP™ Blocking Ligand, the solution will be bright pink.

2. Carefully remove the Resin Equilibration Buffer from the HaloLink™ Resin that you prepared in Step 4 of Phase 1. Add 600µl of the HaloCHIP™ experimental sample or either control sample to separate tubes of the HaloLink™ Resin.

3. Incubate the samples with constant mixing using a tube rotator for 2–3 hours at room temperature.

   **Note:** Ensure constant mixing since settling of the HaloLink™ Resin will reduce binding efficiency.

4. Centrifuge the samples at 800 × *g* for 2 minutes at room temperature.

5. Remove and discard the supernatant.

   **Optional:** If you are analyzing the binding efficiency to the HaloLink™ Resin, save the supernatant (unbound material) and continue on to Section 5.D.

6. Add 1ml of Mammalian Lysis Buffer to the resin and mix thoroughly. Centrifuge at 800 × *g* for 2 minutes. Carefully remove and discard the supernatant.

7. Add 1ml of Nuclease-Free Water and mix thoroughly. Centrifuge at 800 × *g* for 2 minutes. Carefully remove and discard the supernatant.

8. Repeat Step 7.

9. Add 1ml of High Salt Wash Buffer and mix thoroughly. Incubate at room temperature for 5 minutes, mixing using a tube rotator. Centrifuge at 800 × *g* for 2 minutes. Carefully remove and discard the supernatant.

   **Optional:** If you observe nonspecific DNA capture, we recommend adding one LiCl wash after the high salt wash. See Section 7 for LiCl buffer composition.

## 5.A. HaloCHIP™ System Protocol (continued)

### Phase 3. Capture and Release of DNA (continued)

10. Add 1ml of Nuclease-Free Water and mix thoroughly. Centrifuge at 800 × *g* for 2 minutes. Carefully remove and discard the supernatant.

11. Repeat Step 10.

12. Add 1ml of Nuclease-Free Water and mix thoroughly. Incubate for 5 minutes at room temperature mixing using a tube rotator. Centrifuge for 2 minutes at 800 × *g*, and carefully remove and discard the supernatant.

13. Add 300µl of Reversal Buffer to each tube with the HaloLink™ Resin and gently mix by pipetting. Incubate at 65°C for a minimum of 6 hours but not longer than overnight to reverse the crosslinks.

14. Centrifuge the HaloLink™ Resin at 800 × *g* for 2 minutes. Transfer the supernatant containing the released DNA to a new tube.

15. We recommend that the eluted DNA can be further purified using a PCR product purification kit in order to reduce the salt and concentrate the DNA prior to use in an amplification reaction.

    **Caution:** Avoid using DNA purification minicolumns that do not effectively purify smaller fragments (100–500bp).

## 5.B. Analysis of Recovered DNA

The recovered DNA is compatible with all amplification methods, including standard and quantitative PCR. The control reaction outlined in the protocol should be subjected to similar analysis and will show levels of background DNA. If standard PCR analysis is employed, it is important that the PCR be performed within the linear amplification phase. The appropriate number of cycles must be determined experimentally. If the number of cycles is too high, the background signal from the control will be artificially high, resulting in a reduced signal-to-noise ratio.

### Scale-up of HaloCHIP™ Reactions

A single HaloCHIP™ reaction yields approximately 1–10ng of DNA. To obtain more DNA, increase the number of HaloCHIP™ reactions performed, following the protocol for each HaloCHIP™ reaction, and pool purified DNA at the end of the process. This DNA can then be lyophilized to the desired concentration for other downstream analysis.

### 5.C. Assay for Optimizing Sonicated DNA Fragment Size

If sonication conditions need to be optimized, see Section 4. The following assay can be used to determine the sonicated DNA fragment size.

1. Follow the HaloCHIP™ protocol (Section 5.A) to the end of Phase 2, Step 15.
2. Following sonication, incubate the lysate at 95°C for 15 minutes to reverse the crosslinks.
3. Centrifuge the sample at 14,000 × *g* for 5 minutes at 4°C
4. Purify the DNA in the supernatant using a standard method for purifying PCR products.

   **Caution:** Avoid PCR purification Systems that do not effectively purify smaller fragments.
5. Load 20µl of the purified DNA onto a 1–2% agarose gel, and determine the fragment size. The goal is to obtain a smear of DNA of 500–1,500bp (Figure 2). If the majority or "smear" of the fragment sizes are not within this range, increase or decrease the output on the sonicator and the number of pulses or cycles during sonication.

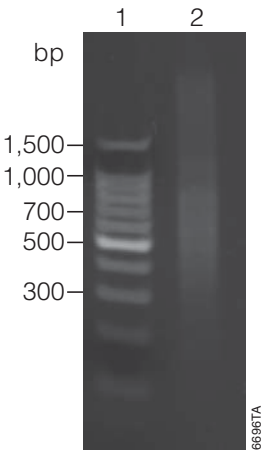


**Figure 2. Sonicated DNA.** HeLa cells transfected with p65-HaloTag® protein were crosslinked, lysed, and sonicated following the HaloCHIP™ System protocol to Phase 2, Step 15, then analyzed using the protocol in Section 5.C. Lane 1, Promega BenchTop 100bp DNA Ladder (Cat.# G8291); lane 2, smear of DNA fragments between the range of 500–1500bp, which is the target size for sonication.

### 5.D. Analysis of HaloTag® Fusion Protein Binding Efficiency to HaloLink™ Resin

This protocol is optional; however, we strongly recommend that you analyze the efficiency of HaloTag® fusion protein binding before completing the entire protocol. Aliquots of the experimental and control samples can be analyzed with this gel-based assay.

**Before you Begin**

Prepare 50–100µl of 50µM HaloCHIP™ Blocking Ligand by diluting an aliquot of the 5mM HaloCHIP™ Blocking Ligand 1:100 in DMSO. Store in amber tubes at –20°C.

1. Remove 10µl from each sample at the following points in the protocol: Section 5.A, Phase 3, Step 2 (input material); Section 5.A, Phase 3, Step 7 (unbound material).
2. Add 20µl of water to each sample and mix.
3. Add 1µl of 50µM HaloCHIP™ Blocking Ligand, and mix thoroughly.
4. Incubate for 15 minutes at room temperature in the dark.
5. Add 10µl of 4X SDS-PAGE gel loading buffer, and heat to 70–95°C for 5 minutes.
6. Analyze 10–15µl of each reaction by SDS-PAGE.
7. Run the gel until the dye front is near the bottom of the gel.
8. Quantitate the amount of input material and unbound material using a fluorescent detection scanner (excitation: 555nm; emission: 585nm). The HaloTag® fusion protein will appear as a fluorescent band on a gel when a fluorescence scanner is used to view the gel. Quantitate the amount of input material and the amount of unbound material by determining the fluorescent signal of the HaloTag® fusion protein in the two samples. The amount of unbound material should be less than 40% of the input material. If the unbound material exceeds 40% of the input material, then the HaloTag® fusion protein was not bound efficiently. This could be due to inactivation of HaloTag® fusion protein. See Section 6, Troubleshooting.

### 5.E. Cytoplasmic Lysis

Cytoplasmic lysis removes the cytoplasmic protein fraction and allows you to proceed only with the nuclear protein fraction. If the majority of your HaloTag® fusion protein is in the nucleus, you may want to remove the cytoplasmic fraction before binding to the HaloLink™ Resin. This will prevent the cytoplasmic protein from competing with the nuclear protein:DNA complexes for binding to the HaloLink™ Resin.

1. Prepare the appropriate volume of cytoplasmic lysis buffer (Section 7).
2. Resuspend the cell pellet (Section 5.A, Phase 2, Step 12) in 500µl of cytoplasmic lysis buffer.
3. Incubate on ice for 20 minutes.
4. Lyse the cells using mechanical disruption (as described in Section 5.A, Phase 2, Step 14).
5. Centrifuge at 2,000 × *g* for 5 minutes at 4°C.
6. Carefully remove the supernatant and save the nuclei cell pellet. Proceed to Section 5.A, Phase 2, Step 13.

## 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 binding of the HaloTag® fusion protein to the HaloLink™ Resin | Protein inactivation due to:<br>• Excessive crosslinking. Perform crosslinking using a lower concentration of formaldehyde (e.g., 0.5%) or reduce time and temperature of crosslinking.<br>• Inefficient quenching. Increase quenching by extending the time or concentration of glycine treatment.<br>• Oversonication and overheating during sonication. Decrease sonication time and output settings. Keep all samples on ice during sonication to avoid denaturation of the HaloTag® fusion protein.<br>• Inefficient lysis. Increase incubation time in Mammalian Lysis Buffer (Section 5.A, Phase 2, Step 13) or increase sonication time.<br>• Use of protease inhibitor cocktails. A protease inhibitor cocktail is not required for the system. However, if adding a protease inhibitor cocktail is desired, we recommend BaculoGold™ Protease Inhibitor Cocktail (BD Biosciences, Cat.# 554779). Avoid cocktails of unknown composition or those known to contain AEBSF (4-(2-aminoethyl benzenesulfonyl fluoride hydrocholoride). AEBSF will significantly reduce binding efficiency.<br>• Presence of contaminants (ethanol or DMSO). Avoid adding more than 2% final concentration of ethanol or DMSO (typically found in protease inhibitor cocktails) to the cell lysate before HaloLink™ Resin binding. |
| | Suboptimal binding conditions.<br>• Increase the time allowed for binding of HaloTag® fusion protein to the HaloLink™ Resin.<br>• Adjust binding temperature within the range of 4°C and room temperature. |

| Symptoms | Causes and Comments |
|---|---|
| Inefficient binding of the HaloTag® fusion protein to the HaloLink™ Resin (continued) | High concentrations of IGEPAL® CA-630 might inhibit binding of HaloTag® fusion protein.<br>• Reduce IGEPAL® CA-630 concentration in the Resin Equilibration Buffer. The effective range of IGEPAL® CA-630 is 0.01%–0.1%. |
| Too much cytoplasmic protein present in the lysate | Perform optional cytoplasmic lysis step (Section 5.E). |
| Unable to detect DNA after reversal of crosslinking and purification | See Causes and Comments for "Inefficient binding of the HaloTag® fusion protein to the HaloLink™ Resin." |
| | Loss of DNA during final purification.<br>• If using a PCR product purification kit, determine if small DNA fragments can be efficiently purified.<br>• Omit the DNA purification step. Instead perform the reversal in a smaller volume (i.e., 100µl of Reversal Buffer), and dilute the sample before PCR. We recommend diluting the reaction five- to sixfold to decrease the salt concentration to 50mM. |
| | Drying of the HaloLink™ Resin during reversal. To prevent evaporation of liquid from the HaloLink™ Resin during the reversal step, use a larger volume or an enclosed system. |
| | Inadequate reversal time. Optimize the reversal time (Section 5.A, Phase 3, Step 13); the optimal time is 6–18 hours. |
| | Inefficient binding of the HaloTag® fusion protein to DNA.<br>• Assay for physiological function of HaloTag® fusion protein in cells. Decrease the amount of HaloTag® fusion vector used during transfection, and add a carrier vector to adjust the DNA concentration to that recommended by the transfection reagent's manufacturer.<br>• Increase formaldehyde concentration to 1% and/or crosslinking time to 15 minutes. |
| | Low or no expression of HaloTag® fusion protein.<br>• Optimize the transfection conditions to increase expression levels. |

(continued)

## 6. Troubleshooting (continued)

| Symptoms | Causes and Comments |
|---|---|
| High background (high amount of DNA detected in the control sample and/or presence of additional bands) | Nonspecific interactions.<br>• Increase washing stringency. Increase the number and/or volume of washes; include additional washes with TE buffer or Nuclease-Free Water. This is especially important when scaling up the experiment.<br>• Equilibrate the HaloLink™ Resin in 0.1% IGEPAL® CA-630 for at least 2 hours prior to adding the lysate.<br>• Add BSA (0.1–1mg/ml) to the lysates and wash buffers (use Blot-Qualified BSA; e.g., Cat.# W3841).<br>• Increase incubation time during washing steps.<br>• To increase specificity of recovered DNA, cleave the protein of interest from HaloLink™ Resin using Factor Xa Protease (Cat.# V5581) or ProTEV Plus (Cat.# V6101), depending upon the cleavage site present in the HaloTag® vector prior to crosslinking reversal.<br>• If performing PCR, vary the number of cycles to determine if amplification is in the linear range. There is always a small amount of background DNA in the control sample, which can be overamplified if too many PCR cycles are performed. |
| | Too much HaloLink™ Resin in the reaction may cause higher background. Reduce the volume of resin used. |
| | Inefficient blocking of the HaloTag® fusion protein in the control sample. Increase the incubation time and/or concentration of HaloCHIP™ Blocking Ligand (Section 5.A, Phase 3, Step 3). |
| | Too many PCR cycles. Reduce the number of PCR cycles so that PCR is stopped during the linear amplification phase. The appropriate number of cycles must be determined experimentally. |

| Symptoms | Causes and Comments |
|---|---|
| Low signal-to-noise ratio | See Causes and Comments for "Inefficient binding of the HaloTag® fusion protein to the HaloLink™ Resin." |
| | Loss of DNA during final purification. If using a PCR product purification kit, determine if it can efficiently purify small DNA fragments. |
| | Too many PCR cycles. Reduce the number of PCR cycles so that PCR is stopped during the linear amplification phase. The appropriate number of cycles must be determined experimentally. |

## 7. Composition of Buffers and Solutions

**4X SDS-PAGE gel loading buffer**

| | |
|---|---|
| 240mM | Tris-HCl (pH 6.8) |
| 3mM | bromophenol blue |
| 50.4% | glycerol |
| 400mM | dithiothreitol (DTT) |
| 2% | SDS |

Add DTT just before use.

**cytoplasmic lysis buffer (optional)**

| | |
|---|---|
| 5mM | Tris-HCl (pH 8.0) |
| 85mM | KCl |
| 1% | IGEPAL® CA-630 |

**HaloCHIP™ Blocking Ligand**

5mM fluorescent TMR HaloTag® Blocking Ligand in DMSO (excitation: 555nm; emission: 580nm)

**HaloLink™ Resin Equilibration Buffer**

| | |
|---|---|
| 1X | TE Buffer (pH 8.0) |
| 0.1% | IGEPAL® CA-630 |

Mix TE Buffer (pH 8.0; provided) and IGEPAL® CA-630.

**Note:** IGEPAL® CA-630 is added to prevent nonspecific binding and sticking of the resin to the sides of the tube. (IGEPAL® CA-630 is an NP40 analog). The effective range of IGEPAL® CA-630 is 0.01% to 0.1%. Prepare a 10% stock solution of IGEPAL® CA-630 in TE Buffer or water and make further dilutions from this solution.

**Important:** HaloLink™ Resin Equilibration Buffer and 10% IGEPAL® CA-630 stock solution should be prepared fresh. Do not store these solutions longer than one week after they are prepared. Store at room temperature.

**LiCl Wash Buffer (optional)**

| | |
|---|---|
| 100mM | Tris-HCl (pH 8.0) |
| 500mM | LiCl |
| 1% | IGEPAL® CA-630 |
| 1% | sodium deoxycholate |

**HaloLink™ Resin**

**Composition:** HaloTag® Binding Ligand is covalently attached to the surface of Sepharose® beads.

**Particle size:** 45–165 microns.

**Concentration:** HaloLink™ Resin is supplied as a 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.

**High Salt Wash Buffer**

| | |
|---|---|
| 50mM | Tris-HCl (pH 7.5) |
| 700mM | NaCl |
| 1% | Triton® X-100 |
| 0.1% | sodium deoxycholate |
| 5mM | EDTA |

**Mammalian Lysis Buffer**

| | |
|---|---|
| 50mM | Tris-HCl (pH 7.5) |
| 150mM | NaCl |
| 1% | Triton® X-100 |
| 0.1% | sodium deoxycholate |

**Reversal Buffer**

| | |
|---|---|
| 10mM | Tris-HCl (pH 8.0) |
| 1mM | EDTA |
| 300mM | NaCl |

**TE Buffer (pH 8.0)**

| | |
|---|---|
| 10mM | Tris-HCl (pH 8.0) |
| 1mM | EDTA |

## 8. References


1. Solomon, M.J., Larsen, P.L. and Varshavsky, A. (1988) Mapping protein-DNA interactions in vivo with formaldehyde: Evidence that histone H4 is retained on a highly transcribed gene. *Cell* **53**, 937–47.
2. *HaloTag® Technology: Focus on Imaging Technical Manual* #TM260, Promega Corporation.
3. Los, G.V. *et al.* (2005) HaloTag™ Interchangeable Labeling Technology for cell imaging, protein capture and immobilization. *Promega Notes* 89, 2–6.


## 9. Related Products

| Product | Size | Cat.# |
|---|---|---|
| HaloLink™ Resin | 1.25ml | G1912 |
| | 2.5ml | G1913 |
| | 10ml | G1914 |
| | 25ml | G1915 |
| Magne® HaloTag® Beads | 1ml | G7281 |
| | 5 × 1ml | G7282 |
| HaloTag® Protein Purification System | 25ml | G6280 |
| HaloTag® Alexa Fluor® 488 Ligand | 30µl | G1001 |
| HaloTag® Oregon Green® Ligand | 30µl | G2801 |
| HaloTag® TMR Ligand | 30µl | G8251 |
| HaloTag® diAcFAM Ligand | 30µl | G8272 |
| HaloTag® Coumarin Ligand | 30µl | G8581 |
| HaloTag® Biotin Ligand | 30µl | G8281 |
| HaloTag® PEG-Biotin Ligand | 30µl | G8591 |
| HaloTag® Amine (04) Ligand | 5mg | P6741 |
| HaloTag® Succinimidyl Ester (04) Ligand | 5mg | P6751 |
| HaloTag® Thiol (04) Ligand | 5mg | P6761 |
| HaloTag® Iodoacetamide (04) Ligand | 5mg | P6771 |
| HaloTag® Succinimidyl Ester (02) Ligand | 5mg | P1691 |
| HaloTag® Amine (02) Ligand | 5mg | P6711 |
| HaloTag® Iodoacetamide (02) Ligand | 5mg | P1681 |
| Blot-Qualified BSA | 10g | W3841 |

## 9. Related Products (continued)

### HaloTag® Flexi® Vectors

| Product | Size | Cat.# |
|---|---|---|
| pFC14A HaloTag® CMV Flexi® Vector | 20µg | G9651 |
| pFC14K HaloTag® CMV Flexi® Vector | 20µg | G9661 |
| pFC15A HaloTag® CMV*d1* Flexi® Vector | 20µg | G1611 |
| pFC15K HaloTag® CMV*d1* Flexi® Vector | 20µg | G1601 |
| pFC16A HaloTag® CMV*d2* Flexi® Vector | 20µg | G1591 |
| pFC16K HaloTag® CMV*d2* Flexi® Vector | 20µg | G1571 |
| pFC17A HaloTag® CMV*d3* Flexi® Vector | 20µg | G1551 |
| pFC17K HaloTag® CMV*d3* Flexi® Vector | 20µg | G1321 |
| pFN21A HaloTag® CMV Flexi® Vector | 20µg | G2821 |
| pFN21K HaloTag® CMV Flexi® Vector | 20µg | G2831 |
| pFN22A HaloTag® CMV*d1* Flexi® Vector | 20µg | G2841 |
| pFN22K HaloTag® CMV*d1* Flexi® Vector | 20µg | G2851 |
| pFN23A HaloTag® CMV*d2* Flexi® Vector | 20µg | G2861 |
| pFN23K HaloTag® CMV*d2* Flexi® Vector | 20µg | G2871 |
| pFN24A HaloTag® CMV*d3* Flexi® Vector | 20µg | G2881 |
| pFN24K HaloTag® CMV*d3* Flexi® Vector | 20µg | G2981 |
| HaloTag® Flexi® Vectors—CMV Deletion Series Sample Pack | 9 × 2µg | G3780 |
| 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 |

## 10. Summary of Changes

The following changes were made to this document:
Patent statements were updated.

[a]BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE LABEL LICENSE. If researcher is not willing to accept the terms of this label license, and the product is unused, Promega will accept return of the unused product and provide researcher with a full refund.

Researcher may use this product for research use only; no transfer or commercial use of this product is allowed. Commercial use means any and all uses of this product by a party in exchange for consideration, including, but not limited to (1) use in further product manufacture; (2) use in provision of services, information or data; and (3) resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research. With respect to any uses outside this label license, including any commercial, diagnostic, therapeutic or prophylactic uses, please contact Promega for supply and licensing information. PROMEGA MAKES NO REPRESENTATIONS OR WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED, INCLUDING FOR MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE WITH REGARDS TO THE PRODUCT. The terms of this label license shall be governed under the laws of the State of Wisconsin, USA.

[b] U.S. Pat. Nos. 7,429,472, 7,888,086, 8,202,700 and other patents pending.



Flexi and HaloTag are registered trademarks of Promega Corporation. HaloCHIP and HaloLink are trademarks of Promega Corporation.

Alexa Fluor and Oregon Green are registered trademarks of Molecular Probes, Inc. BaculoGold is a trademark of Becton, Dickinson and Company. IGEPAL is a registered trademark of Rhone-Poulenc AG Co. Nonidet is a registered trademark of Shell International Petroleum Company, Ltd. Sepharose is a registered trademark of GE Healthcare Bio-sciences. Triton is a registered trademark of Union Carbide Chemicals & Plastics Technology Corporation. Tween is a registered trademark of ICI Americas, Inc.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

**Promega Corporation** · 2800 Woods Hollow Road · Madison, WI 53711-5399 USA
Toll Free in USA 800-356-9526 · Phone 608-274-4330 · Fax 608-277-2516 · **www.promega.com**
Printed in USA. Part# TM075
Revised 12/15