HaloTag® Mammalian Protein Detection and Purification Systems

INSTRUCTIONS FOR USE OF PRODUCTS G6790, G6795 AND G6799.

Quick PROTOCOL

HaloTag® Purification — Quick Purification Protocol from 2 × 10^8 cells

Lyse

1. Resuspend the cell pellet in 5ml HaloTag® Purification Buffer.
2. Add 100µl of 50X Protease Inhibitor Cocktail.
3. Sonicate on ice (avoid overheating as this will inhibit binding).
   Note: For other lysis methods please refer to TM348.
4. Harvest cell lysate at 4°C (10,000 × *g* for 15 minutes); collect supernatant.

Equilibrate Resin

5. Transfer 600µl of HaloLink™ Resin slurry to a tube.
6. Centrifuge at 1,500 × *g* for 5 minutes; discard the supernatant.
7. Wash the resin five times:
   a. Add 5ml of HaloTag® Purification Buffer; mix for 5 minutes.
   b. Centrifuge at 1,500 × *g* for 5 minutes; discard the supernatant.

Bind

8. Add the cell lysate to the equilibrated resin.
9. Incubate for 90 minutes at room temperature (22–25°C) with constant mixing.
10. Centrifuge at 1,500 × *g* for 5 minutes; remove supernatant, save as sample flowthrough.

Wash

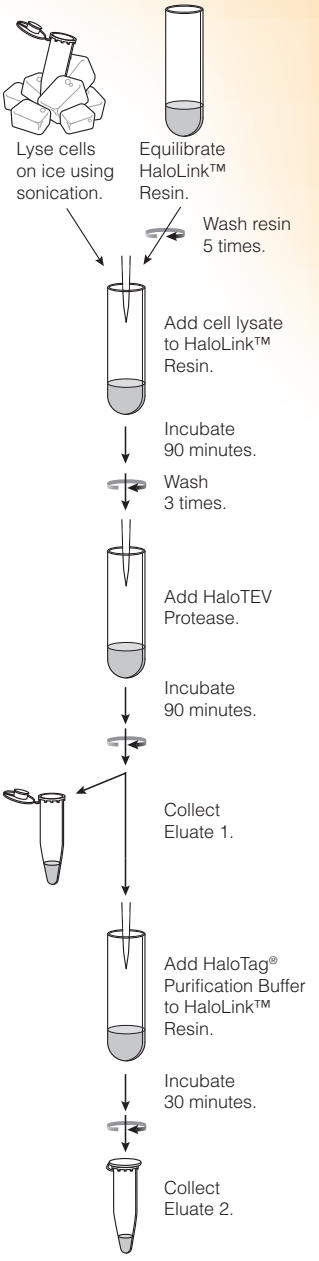
11. Wash the resin three times:
    a. Add 5ml of HaloTag® Purification Buffer; mix at room temperature for 10 minutes.
    b. Centrifuge at 1,500 × *g* for 5 minutes; discard the supernatant.

Cleave

12. Combine 9µl of HaloTEV Protease with 291µl of HaloTag® Purification Buffer.
13. Add the cleavage solution to the resin; incubate at room temperature (22–25°C) for 90 minutes with constant mixing.

Elute

14. Centrifuge at 1,500 × *g* for 5 minutes; collect the supernatant (Elution 1).
15. Add 300µl HaloTag® Purification Buffer to the resin; mix for 30 minutes at room temperature.
16. Transfer the resin into the spin column; centrifuge at 10,000 × *g* for 15 seconds; collect Elution 2.
17. Centrifuge Elution 1 and Elution 2 at 10,000 × *g* for 1 minute, and transfer to clean tubes.



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Protocol information in Technical Manual #TM348, available online at ***www.promega.com***

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Quick PROTOCOL

HaloTag® Fusion Protein Detection

Fluorescent Labeling

Fluorescent labeling of HaloTag® fusion protein with the HaloTag® TMRDirect™ Ligand provides a rapid and convenient method to monitor protein expression and follow the purification efficiency.

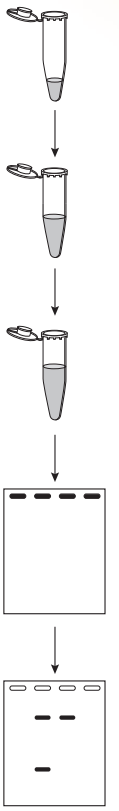
1. Dilute the HaloTag® TMRDirect™ Ligand stock solution (100µM) twofold in DMSO to make a 50µM working solution. Store protected from light, at –20°C.

   Note: Alternatively, the stock solution can be prepare in PBS, but cannot be stored.

2. Combine 10µl of lysate containing the HaloTag® fusion protein with 19µl of HaloTag® Protein Purification Buffer and 1µl of 50µM HaloTag® TMRDirect™ Ligand.

   Note: The equivalent amount of unbound fraction can be added in place of the lysate.

3. Incubate at room temperature for 15 minutes protected from light.
4. Add 10µl of 4X SDS gel loading buffer and heat at 70°C for 3 minutes.
5. Load 10µl onto an SDS-polyacrylamide gel.
6. Following electrophoresis, scan the gel on a fluorescence imager such as the Typhoon® (excitation 532nm, emission 580nm), and quantitate band intensities.



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See additional protocol information in Technical Manual #TM348, available online at ***www.promega.com***

For further information regarding HaloTag® labeling refer to the HaloTag® Technology: Focus on Imaging Technical Manual *#TM260 or visit:* ***www.promega.com.***

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