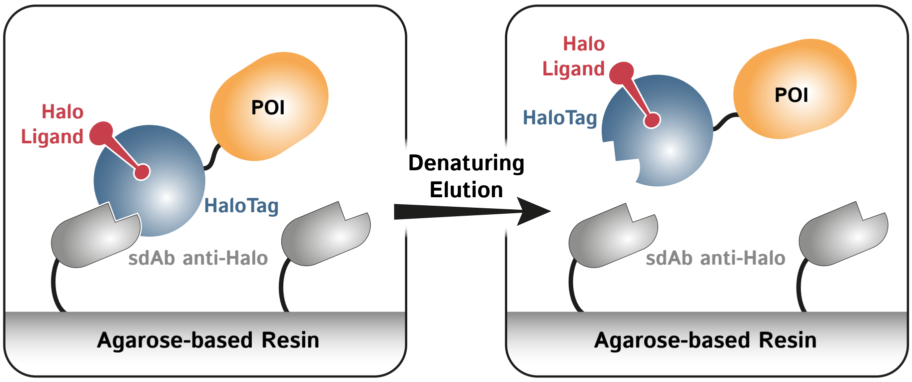
Halo Selector Resins for Analytical Immunoprecipitation of Halo Fusion Proteins

A. Introduction

The **HaloTag®(1) technology** is a widely used and highly versatile platform in molecular and cellular biology, enabling detailed analyses of protein function, dynamics, and molecular interactions. It is based on a genetically engineered haloalkane dehalogenase derived from bacteria, which has been modified to form a specific and stable covalent bond with synthetic Halo ligands(2). These ligands can be functionalized with a variety of chemical groups, including fluorescent dyes and biotin, thereby facilitating a broad range of applications such as live-cell imaging and protein labeling.

NanoTag's **Halo Selectors** are specialized **affinity resins** designed for the efficient purification and enrichment of Halo-tagged proteins. They feature a **high-affinity single-domain antibody (sdAb)** that specifically recognizes the HaloTag®. Binding of Halo fusion proteins to Halo Selectors typically does not interfere with the interaction of the HaloTag® with its ligands, thereby preserving the functional integrity of the fusion protein during purification or assay workflows.



Halo Selector Resins are available in both **agarose** or **magnetic agarose bead formats**. The site-directed and chemically stable immobilization of target-specific sdAbs ensures high capacity (up to >150 µM target protein, e.g., ~5 mg of HaloTag® per mL of resin) and optimal accessibility of available binding sites. At the same time, minimal leakage of sdAbs is observed, even under harsh denaturing and/or reducing conditions. Due to their combination of high capacity, minimal leakage and extraordinarily low non-specific protein adsorption, Halo Selector Resins enable clean and highly specific immunoprecipitations.

NanoTag's Halo Selector Resins are broadly compatible not only with physiological buffers and most common lysis/washing buffers, but also with high stringency buffers, detergents, and reducing agents. The resins can therefore be used in customized applications employing a wide variety of buffer compositions and pH values.

Formats and Applications

Halo Selector Resins are available in two alternative formats:

Product Name	Cat. No.	Bead Format	Handling Options
Halo Selector 2 mL, 50 % slurry	N5010	4 % cross-linked agarose	Recommended for analytical immunoprecipitations using MiniSpin columns
Halo Selector, magnetic 2 mL, 50 % slurry	N5015	4 % cross-linked magnetic agarose	Optimized for magnetic bead separation protocols

Capacity

The effective binding capacity of Halo Selector Resins depends on the hydrodynamic properties of the proteins being purified – such as their size and shape. Due to size exclusion effects, the capacity may be reduced for large proteins or protein complexes. The table below shows the experimentally determined capacity for selected example proteins.

Protein	Molecular Weight	Binding Capacity
HaloTag	33 kDa	5 mg/mL (150 µM)
mNeonGreen-HaloTag	70 kDa	>4.0 mg/mL

B. Protocols

I. Required Materials (not included)		
Buffers	Lysis Buffer[a]	PBS or TBS (other formulations possible)[a]
	Washing Buffer[a]	PBS or TBS (other formulations possible)[a]
	2x SDS Sample Buffer	125 mM Tris/HCl pH 6.8, 4% SDS, 100 mM DTT, 1 M sucrose, 0.1 mg/mL bromophenol blue
Columns and Tubes		
Optional: Magnetic rack		

[a] Buffers can be adjusted across a wide range of conditions. See the buffer compatibility chart on page 6.

II. Analytical scale purifications using non-magnetic Halo Selector Resin

We recommend using MiniSpin Columns (Cat. No. A1001) for analytical scale immunoprecipitation experiments using non-magnetic Halo Selector Resins

1. Prepare cell lysates (0.2 to 1.5 mL volume) from cells expressing a Halo fusion protein according to established protocols. For mammalian cells, we recommend using 10^6-10^8 cells per experiment.

2. Clear lysate by centrifugation for 10 min at > 14000 x g and 4 °C.
   Repeat clearing step if clouding of the sample is observed.
   Take sample for further analysis (Input fraction).

3. Equilibrate the Halo Selector Resin:
   a. Resuspend Halo Selector Resin.
   b. Transfer 20 µL slurry (10 µL packed beads) into a clean 1.5 mL reaction tube.
   c. Add 1 mL Lysis Buffer.
   d. Centrifuge for 1 min at 1000 x g and carefully remove supernatant.
   e. Repeat steps 3c-3d.

4. Add cleared lysate from step 2 to the equilibrated Halo Selector Resin from step 3.

5. Incubate 1 h at 4 °C with head-over-tail rotation.

6. Sediment beads by centrifugation for 1 min at 1000 x g and 4 °C.
   Take sample from supernatant for further analysis (Non-bound fraction).

7. Washing:
   a. Carefully remove supernatant.
   b. Resuspend beads in 1 mL Lysis Buffer.
   c. Centrifuge for 1 min at 1000 x g.
   d. Remove supernatant.
   e. Repeat steps 7b-7d twice.

8. Transfer:
   a. Remove bottom plug from MiniSpin Column. Place column in 2 mL reaction tube.
   b. Resuspend beads in 200 µL Lysis Buffer and transfer suspension to MiniSpin Column.
   c. Wash out remaining beads sticking to the tube with 200 µL Lysis Buffer.
   Transfer the suspension to the MiniSpin Column.
   d. Centrifuge for 1 min at 1000 x g and discard flow-through.

9. Washing:
   a. Add 400 µL Lysis Buffer.
   b. Centrifuge for 1 min at 1000 x g and discard flow-through.
   c. Repeat steps 9a-9b.

10. Wash with 400 µL TBS, centrifuge for 1 min at 1000 x g.

11. Attach bottom plug and place MiniSpin Column in a clean 1.5 mL reaction tube.

(Protocol continued on next page)

12. Denaturing elution using SDS Sample Buffer

> **!** Halo-tagged target proteins can efficiently be eluted using SDS Sample Buffer at elevated temperatures. Denaturing elution will in general not be compatible with protein functionality and is therefore explicitly recommended for analytical applications only. Elution at acidic pH is not recommended.

a. Add 2.5 resin volumes (RV) of 2x SDS Sample Buffer pre-warmed to ~60 °C to the resin.
b. Close the MiniSpin Column by attaching the lid and bottom plug.
c. Incubate for 5 min at 80-90 °C with subtle shaking.
d. Open lid and remove the bottom plug and collect the eluate by centrifugation 1 min at 1000 x g.
e. Attach bottom plug and repeat steps a-d.
f. Combine eluates.

13. Take sample from elution fractions for further analysis (Elution fraction).

14. Analyze collected samples by SDS-PAGE.

III. Analytical purifications using magnetic Halo Selector Resin

1. Prepare cell lysates (0.2 to 1.5 mL volume) from cells expressing a Halo fusion protein according to established protocols. For mammalian cells, we recommend using 10^6-10^8 cells per experiment.

2. Clear lysate by centrifugation for 10 min at > 14000 x g and 4 °C.
   Repeat clearing step if clouding of the sample is observed.
   Take sample for further analysis (Input fraction).

3. Equilibrate the magnetic Halo Selector Resin:
   a. Resuspend Halo Selector Resin.
   b. Transfer 20 µL slurry (10 µL packed beads) into a clean 1.5 mL reaction tube.
   c. Add 1 mL Lysis Buffer.
   d. Centrifuge for 1 min at 1000 x g and carefully remove supernatant.
   e. Repeat steps 3c-3d.

4. Add cleared lysate from step 2 to the equilibrated magnetic Halo Selector Resin from step 3.

5. Incubate 1 h at 4 °C with head-over-tail rotation.

6. Collect beads by placing the tube in a magnetic rack
   Take sample from supernatant for further analysis (Non-bound fraction).

7. Washing:
   a. Carefully remove supernatant.
   b. Resuspend beads in 1 mL Lysis Buffer and incubate 1-2 min head-over-tail.
   c. Collect beads by placing the tube in a magnetic rack.
   d. Remove supernatant.
   e. Repeat steps 7b-7d three times.
   f. Resuspend beads in 1 mL Lysis Buffer and transfer suspension into fresh 1.5 mL tube.
   Note: This step is essential as generally a lot of contaminant proteins stick to the walls of the tube used during the binding step!
   g. Wash beads twice as described in steps 7b-7d.
   h. Wash with 1 mL TBS and collect beads on magnetic rack.
   i. Remove the supernatant completely.

8. Denaturing elution using SDS Sample Buffer

> **!** Halo-tagged target proteins can efficiently be eluted using SDS Sample Buffer at elevated temperatures. Denaturing elution will in general not be compatible with protein functionality and is therefore explicitly recommended for analytical applications only. Elution at acidic pH is not recommended.

   a. Add 2.5 resin volumes (RV) of 2x SDS Sample Buffer pre-warmed to ~60 °C to the resin.
   b. Incubate for 5 min at 80-90 °C with subtle shaking.
   c. Sediment the beads by placing on a magnetic rack.
   d. Collect the liquid phase (eluate).
   e. Repeat steps a-d.
   f. Combine eluates.

9. Take sample from elution fractions for further analysis (Elution fraction).

10. Analyze collected samples by SDS-PAGE.

C. Appendix

I. Buffer compatibility

Please note that the buffer compatibility of Halo Selector Resins differs for binding and washing steps, respectively. In general, Halo Selector Resins are more robust when charged with Halo-tagged target proteins. Therefore, it is often possible to perform highly stringent washing steps under conditions incompatible with an initial binding.

Not all reagent combinations are compatible and some may exhibit more than additive – i.e., synergistic or non-linear – effects. For example, such effects might be expected when combining denaturing agents (including denaturing detergents like SDS) with high concentrations of reducing agents.

Class	Reagent	During Washing	Compatibility[b]
Salt	NaCl	4 M	+++
	$MgSO_4$	1 M	+++
Denaturing agents	Urea	4 M	+++
	Guanidin-HCl	2 M	–
Detergents	Triton-X 100	1 %	+++
	DDM	1 %	+++
	CHAPS	1 %	+++
	Desoxycholate	0.1 %	+++
	SDS	0.1 %	–
Reducing agents	DTT	100 mM	+++

[b] The compatibility was graded according to the amount of target protein remaining on the resin after two washing steps (5 min at RT) in buffer containing the indicated reagent:
– incompatible, protein unfolding and/or complete loss of target protein
+++ compatible; no obvious losses during washing steps

II. References

(1) HaloTag® is a registered trademark of Promega Corporation.

(2) Los GV, Encell LP, McDougall MG, et al. **HaloTag: a novel protein labeling technology for cell imaging and protein analysis.** *ACS Chem Biol*. 2008;3(6):373-382. doi:10.1021/cb800025k

Halo Selector Resins are only for research applications, not for diagnostic or therapeutic use!

For further information concerning this protocol please contact us at info@nano-tag.com