Halo Selector Resins for Analytical Immunoprecipitation of Halo Fusion Proteins.

For the efficient purification and
enrichment of Halo-tagged proteinsusing ab328085 HaloTag-Selector Affinity Resin (4% cross-linked agarose) and ab328086 HaloTag-Selector Affinity Resin (4% cross-linked magnetic agarose).

Storage: Store product at 2-8°C immediately upon receipt. **DO NOT FREEZE.**

Materials Required, Not Supplied

These materials are not included, but will be required to successfully utilize these products:

Lysis buffer - PBS or TBS (other formulations possible)*
Washing buffer - PBS or TBS (other formulations possible)*
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

∆***Note:*** *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.).*

**Buffers can be adjusted across a wide range of conditions. See the buffer compatibility chart on page 2.*

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)

Analytical scale purifications using non-magnetic Halo Selector Resin

We recommend using MiniSpin Columns 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.
12. Denaturing elution using SDS Sample Buffer
∆***Note:*** *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.

Analytical scale 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
∆***Note:*** *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.

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	Compatability*
Salt	NaCl	4 M	+++
	MgSO4	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 nM	+++

** 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

Technical Support

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Version 1 | 2026-09-28

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