Datasheet for ABIN3199244

Rho1D4 MagBeads

3 Images

Go to Product page

Overview

Quantity:	1 mL
Application:	Pull-Down Assay (Pull-Down), Purification (Purif), Separation (Sep)

Product Details

Purpose:	Specific binding and purification of Rho1D4-tagged proteins
Brand:	HighSpec
Specificity:	Affinity to Rho1D4-tagged proteins
Characteristics:	• Dedicated affinity matrix for membrane proteins • Highly tolerant to detergents • Binding capacity <3 mg/mL settled beads • Delivered as a 5 % suspension • Average magnetic agarose bead size: 25 µm
Components:	Affinity Magnetic Agarose
Material not included:	• Lysis Buffer • Wash Buffer • Elution Buffer • Rho1D4 peptide • Ultrasonic homogenizer • Ice bath • Refrigerated centrifuge for 1.5 mL tubes • Refrigerated superspeed or ultracentrifuge capable of 100.000 x g • End-over-end rotator • 2 mL microcentrifuge tubes • Polycarbonate high speed centrifuge tube • Micropipettor and Micropipetting tips

Product Details

	• Magnetic holder for microcentrifuge tubes (for separation of magnetic beads) • pH meter • UV/VIS spectrophotometer • SDS-PAGE reagents and equipment Optional: Western Blot reagents and equipment
Bead Ligand:	Rho1D4
Bead Size:	25 µm

Application Details

Application Notes:	For use with: Rho1D4 affinity column with bound Rho1D4-tagged protein Assay Time Procedure: 2 days (incl. overnight incubation)
Comment:	KD of Rho1D4 antibody to 9 amino acid antigen: ca 20 nM Sample Volume for an assay: 20-60 mL E.coli culture volume or corresponding quantity. Protocol can be scaled up easily.
Protocol:	Purification of Rho1D4-tagged protein using magnetic beads, and pull-down assays
Reagent Preparation:	• Rho buffer: NaH2PO4* 10 mM NaCl* 150 mM Glycerol 10 % (v/v) Protease inhibitor 1x, pH 7.0 Note: Add protease inhibitor directly before use. Depending on the protein, PBS at pH 7.4 may yield better results. • Lysis buffer: 1 x Rho buffer with 1 mg/mL Lysozyme • EW (Equilibration and Wash) buffer: 1 x Rho buffer with appropriate detergent • Elution buffer: 1 x Rho buffer with appropriate detergent and 200 µm Rho1D4 peptide. Note: Always prepare fresh. The recommended concentration of rho1D4 peptide in the elution buffer is 200 µM-1 mM. See the rho1D4 peptide Datasheet for further instructions to reconstitute the lyophilized peptide.
Assay Procedure:	A. Solubilization of the membrane protein 1. Thaw the E. coli cell pellet on ice for 15 min. Optional: Freezing the cell pellet at -20 °C for 30 min prior to incubation at room temperature improves lysis by lysozyme. 2. Resuspend the cell pellet in Lysis Buffer. Use 200 µL Lysis Buffer per 20 mg cell pellet. Pour it into a microcentrifuge tube. 3. If the solution is very viscous, add 3 units Benzonase® per mL E.coli culture volume to the lysis buffer. Alternatively or additionally, sonicate the lysate to improve cell disruption. Note: Keep the lysates on ice to prevent warming. 4. Incubate on an end-over-end shaker at 4 °C for 1 h.

5. Centrifuge the lysate for 15 min at 900 x g and 4 °C to remove cell debris. Note: The supernatant contains the cleared lysate fraction. We recommend to take aliquots of all fractions for SDS-PAGE analysis.
6. Carefully transfer the supernatant to a fresh tube. Centrifuge for 30 min at 7,000 x g and 4 °C to precipitate inclusion bodies. Tip: Analyze the resulting pellet by SDS-PAGE to assess if target protein is present in inclusion bodies. To capture these proteins, we recommend purification via His-tag under denaturing consitions, using HighSpec His Affinity matrices. Alternatively, optimize expression conditions to bring the target protein into the membrane fraction.
7. Carefully transfer the supernatant to a polycarbonate high-speed centrifuge tube and centrifuge at 100,000xg for 1 h at 4 °C.
8. Discard the supernatant and resuspend the pellet in 200 µL EW Buffer. Determine protein concentration and adjust the volume with EW Buffer to a concentration of 5 mg/mL. Note the adjusted volume. Note: The solution contains the total membrane protein fraction.
9. Based on the results from the detergent screen, calculate the amount of detergent needed to solubilize the protein in the adjusted volume. Add the detergent. Note: To determine optimal detergent conditions, refer to the Protocol: "Screening Detergents for Optimal Solubilization and Purification of Membrane Proteins"
10. Transfer the suspension to a clean microcentrifuge tube. Incubate on an end-over-end rotator using the incubation conditions determined in the detergent screen.
11. Transfer the suspension to a polycarbonate high-speed centrifuge tube and centrifuge at 100,000 x g for 1 h at 4 °C.
12. Transfer the supernatant to a fresh microcentrifuge tube and use it in part B of the protocol. Note: The solution contains the solubilized membrane protein fraction.

B. Purification of the membrane protein using Rho1D4 MagBeads

1. Resuspend the HighSpec Rho1D4 MagBeads by vortexing. Transfer 0.2 mL of the 5% suspension into a 1.5 mL microcentrifuge tube. Note: HighSpec Rho1D4 MagBeads are supplied as a 5% suspension with a binding capacity of about 15-20 µg/mL. If you expect a higher protein concentration in your sample, increase the suspension volume.
2. Add 0.5 mL EW Buffer and mix gently. Place the tube on a magnetic microtube stand until the beads are separated and remove the supernatant. Repeat once.
3. Pipet the soluble membrane fraction onto the equilibrated HighSpec Rho1D4 MagBeads and incubate at 4˚C overnight on an end-over-end shaker.
4. Place the tube on a magnetic microtube stand until the beads are separated and remove the supernatant.
5. Remove the tube from the magnet. Add 0.5 mL of Buffer EW and mix gently. Place the tube again on the magnetic microtube stand and allow the beads to separate. Remove the supernatant.
6. Repeat the wash step at least twice.
7. Elute the rho1D4-tagged protein by adding 100 µL Elution Buffer. Close and rotate the column for 1 h at 4°C. Place the tube again on the magnetic microtube stand and allow the beads to separate. Collect the supernatant.

Application Details

8. Repeat step 7 at least 5 times. Collect each eluate in a separate tube and determine the protein concentration of each fraction.
9. Analyze all fractions by SDS-PAGE and Bradford assay or spectrophotometry (280 nm). Note: Do not boil membrane proteins. Instead, incubate samples at 46˚C for 30 min in preparation for SDS-PAGE analysis.
10. Optional: Perform a Western Blot assay using Rho1D4 antibody.

Calculation of Results:	Analyze by SDS-PAGE, Bradford Assay or spectrophotometrically.
Assay Precision:	2 d
Restrictions:	For Research Use only

Handling

Format:	Liquid
Storage:	4 °C

Images

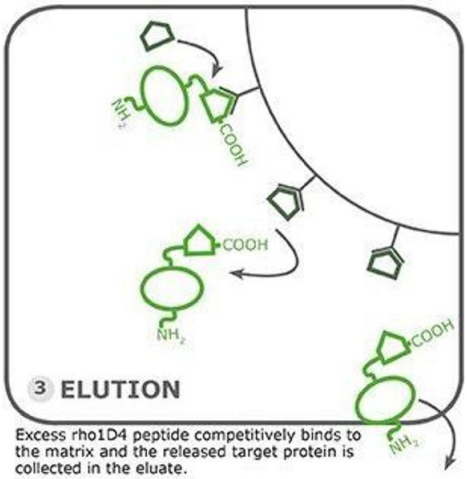
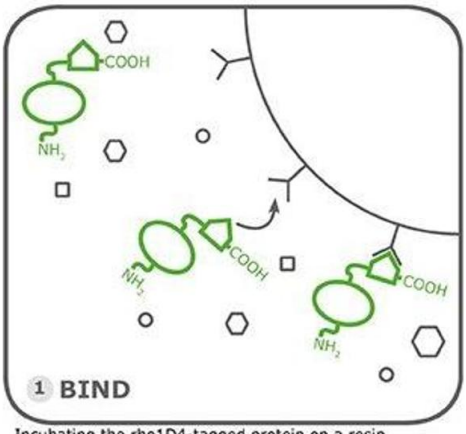
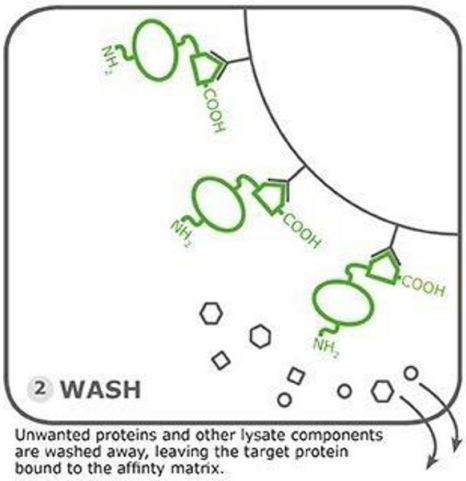


Image 1.



Incubating the rho1D4-tagged protein on a resin loaded with rho1D4 antibody sequesters the target protein from the lysate.

Image 2.



Unwanted proteins and other lysate components are washed away, leaving the target protein bound to the affinty matrix.

Image 3.