Datasheet for ABIN3199243

Rho1D4 Agarose

4 Images

Go to Product page

Overview

Quantity:	1 mL
Application:	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 • Delivered as a 50 % suspension • Average agarose bead size: 40 µm
Components:	Affinity Agarose
Material not included:	• Lysis Buffer • Wash Buffer • Elution Buffer • Rho1D4 peptide • Detergents • Ultrasonic homogenizer • Ice bath • Refrigerated centrifuge for 50 mL tubes (min10,000 x g) and 2 mL tubes • Refrigerated superspeed or ultracentrifuge capable of 100.000 x g • End-over-end rotator • 2 mL microcentrifuge tubes • 15 mL polypropylene tube 50 mL polypropylene tube 50 mL polycarbonate high speed

Product Details

	centrifuge tube (for ultracentrifuge) • Micropipettor and Micropipetting tips • Disposable gravity flow columns with capped bottom outlet, 2 ml • pH meter • UV/VIS spectrophotometer • SDS-PAGE reagents and equipment Optional: Western Blot reagents and equipment
Bead Ligand:	Rho1D4
Bead Size:	40 µm

Application Details

Application Notes:	For use with: E.coli and eukaryotic cell lysates, cell culture supernatants 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: 400-500 mL E.coli culture volume or corresponding quantity.
Protocol:	Purification of Rho1D4-tagged protein in batch gravity flow or on FPLC columns
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 10 mL Lysis Buffer per g cell pellet. Pour it into a 50 mL conical centrifuge 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 conditions, using HighSpec Ni-NTA Agarose. 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 5 mL 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 15 mL polypropylene centrifuge 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 15 mL tube and use it in part B of the protocol. Note: The solution contains the solubilized membrane protein fraction. Resuspend the HighSpec Rho1D4 Agarose by inverting the bottle until the suspension is homogeneous. Transfer 0.2 mL of the 50 % suspension (corresponding to 100 µL bed volume) to a 15 mL conical centrifuge tube. Allow the resin to settle by gravity and remove the supernatant.

B. Purification of the membrane protein using Rho1D4 Agarose

1. Add 1 mL EW Buffer and gently resuspend the slurry to equilibrate the resin. Allow the resin to settle by gravity and remove the supernatant. Tip: Alternatively, resin equilibration can be performed directly in the disposable gravity flow column.
2. Pipet the soluble membrane fraction onto the equilibrated HighSpec Rho1D4 Agarose and incubate at 4˚C overnight on an end-over-end shaker.
3. Transfer the binding suspension to a disposable gravity flow column with a capped bottom outlet. Use EW Buffer to rinse the centrifuge tube and remove resin adhered to the wall.
4. Remove the bottom cap of the column and collect the flow-through.
5. Wash the column with 0.5 mL EW Buffer. Repeat the washing step at least 3 times.
6. Elute the rho1D4-tagged protein by adding 0.2 mL Elution Buffer. Close and rotate the column for 1 h at 4°C. Remove the top and bottom cap of the column and collect the eluate.
7. Repeat step 7 at least 5 times. Collect each eluate in a separate tube and determine the protein concentration of each fraction.

Application Details

	8. 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. 9. 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

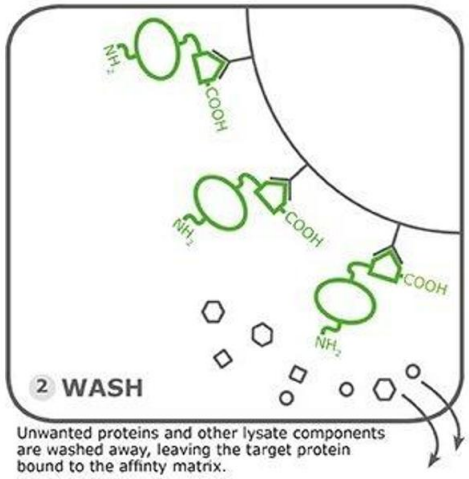


Image 1.

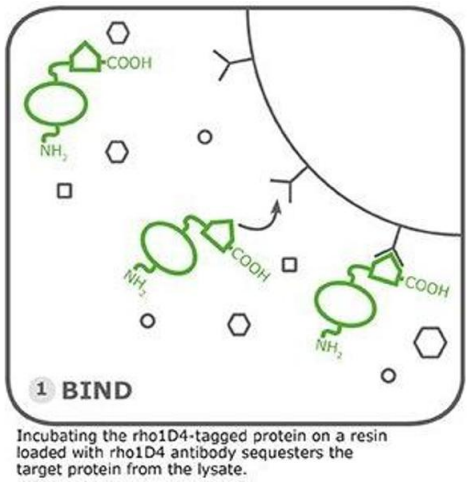
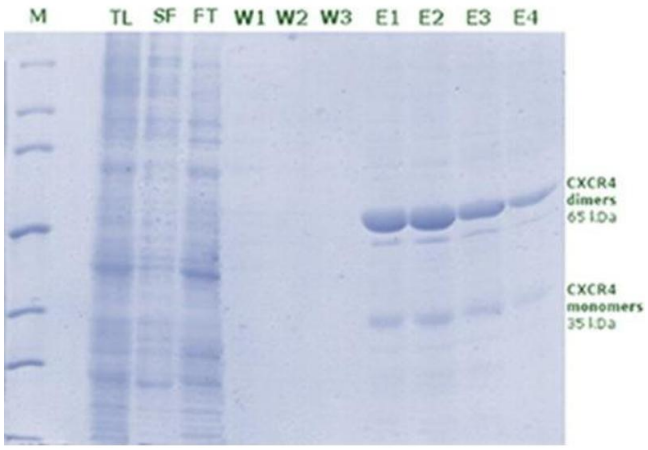


Image 2.

Images



SDS-PAGE

Image 3. Fig. 4: Purification of chemokine receptor 4 (CXCR4) using PureCube Rho1D4 Agarose. Total E.coli lysate (TL) was solubilized with Fos-Choline-14 and the soluble fraction (SF) was incubated on an immunoaffinity column loaded with rho1D4 antibody. Wash fractions (W1-W3) show no detectable loss of target protein. Concentration of eluted CXCR4 in elution fractions (E1-E4) ranged 0.6-1.0 mg/mL as determined spectrophotometrically.

Please check the product details page for more images. Overall 4 images are available for ABIN3199243.