Datasheet for ABIN4886405

MSP1E3D1 Protein (His tag)

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Overview

Quantity:	2 mg
Target:	MSP1E3D1
Origin:	Rat
Source:	Escherichia coli (E. coli)
Protein Type:	Recombinant
Purification tag / Conjugate:	This MSP1E3D1 protein is labelled with His tag.
Application:	Stabilization (STB)

Product Details

Purpose:	Forms nanodisc structures in combination with phospholipids
Specificity:	Stabilization of membrane proteins
Characteristics:	MSP1E3D1 protein, rat sequence, with duplication of helices 4-6. N-terminal 6x His-tag. Resulting nanodisc diameter: ca.12-14 nm The concentration of our recombinant proteins is measured using the absorbance at 280nm. The protein's absorbance will be measured in several dilutions and is measured against its specific reference buffer. The concentration of the protein is calculated using its specific absorption coefficient. We use the Expasy's protparam tool to determine the absorption coefficient of each protein.
Purity:	> 90 %
Components:	Lyophilized MSP protein
Material not included:	• 37 °C incubator

Product Details

- 4 °C incubator
- End-over-end shaker
- Micropipettor and Micropipetting tips
- Gel filtration column
- FPLC instrument with integrated UV detector and fraction collector
- Magnetic stirrer
- Centrifuge for 2 mL microtubes
- 2 mL microtubes
- Palmitoyl-oleoyl-phosphatidylcholine (POPC)
- Dimyristoyl-glycero-phosphocholine (DMPC) or other suitable phospholipid or phospholipid mixture
- Sodium cholate
- Biobeads SM-2
- Single use syringe
- Single use needle
- 0.45 micron filter
- Protein concentrator
- SDS PAGE reagents and equipment
- Optional: Western Blot reagents and equipment and PentaHis Antibody

Target Details

Target:	MSP1E3D1
Abstract:	MSP1E3D1 Products

Application Details

Application Notes:	For use with: Detergent-solubilized membrane protein Assay Time Procedure: 4 days (incl. 3x overnight incubation)
Comment:	Sample Volume for an assay: >1 mg purified membrane protein
Protocol:	Stabilization of Meraner proteins using nanodiscs, especially for immunization of rats
Reagent Preparation:	ND protein buffer: Tris base, pH 7.4 20 mM DMPC protocol: ND lipid buffer: ND protein buffer 1 x, Sodium cholate 100 mM. Note: Always prepare fresh. POPC protocol: ND lipid buffer II: ND protein buffer 1 x, Sodium cholate 200 mM. Note: Always prepare fresh.

Application Details

Assay Procedure:

DMPC: Reconstitution of membrane proteins into nanodisc using his-tagged MSP1D1 protein and DMPC phospholipids

1. Prepare a membrane protein solution containing your target protein of interest. As a starting point, use 200 µL of the protein solution at a concentration of 2-5 mg/mL. Note: Depending on the target protein, required protein concentrations might be up to 20 mg/mL.
2. Determine the molar concentration of target protein solution, using the molecular weight and extinction coefficient e.g. at 280 nm.
3. Calculate the required amount of MSP protein to be 20 times the molar quantity of the target protein.
4. Calculate the required amount of DMPC to be 80 times the molar quantitiy of the MSP protein (or 1,600 times the molar quantity of the target protein). Example: Target protein has a MW of 45,000 Da. Therefore, 500 µg protein in a 200 µL solution corresponds to 11 nmol of target protein in the 1 mL reaction mix. To obtain a 20 fold excess of MSP protein, use 220 nmol of MSP, i.e. 5.57 mg (MW 25.309 g/mol). For an 80 fold excess of phospholipid, use 17.6 µmol or 11.92 mg of DMPC (MW 677.3 g/mol).
5. Resuspend the DMPC in 500 µL ND Lipid Buffer and incubate for 20 min at 37 °C to fully solubilize the phospholipid.
6. Resuspend the MSP protein in 500 µL ND Protein Buffer in a 2 mL microtube. Add the target protein, solubilized in detergent solution. Note: The protein was lyophilized from a solution containing 4 mg/mL protein in 20 mM Tris pH 7.4, 100 mM NaCl, 0.5 mM EDTA.
7. Add the DMPC solution to the MSP and membrane protein solution, and incubate the entire mix at 4 °C for 2 h.
8. During the incubation, equilibrate 2.5 g biobeads or similar adsorbant in ND Protein Buffer according to the manufacturer's instructions. Degas the solution by ultrasound to remove any oxygen solubilized in the biobead solution.
9. Add the protein/DMPC mix to 750 µL of the biobead solution and incubate at 4 °C on an end over end shaker for 8-12 h.
10. Spin the solution at 10-12,000 x g for 2 min, and transfer the supernatant to a fresh tube. Add 750 µL of fresh equilibrated biobead solution and incubate at 4 °C on an end over end shaker for 8-12 h.
11. Repeat step 10 at least one more time to ensure complete detergent removal.
12. Remove the supernatant, and filter the nanodisc mix through a 0.45 micron filter to remove precipitates that might have formed during incubation.
13. Apply the nanodisc mix on a gel filtration column. Apply the mix in several portions if necessary. Monitor absorbance at 280 nm.
14. Collect fractions of ca. 500 µL size, and analyze the samples by SDS PAGE. MSP proteins have an apparent molecular mass of around 20 kDa. Note: Optionally, analysis of fractions in the western blot can be done e.g. with Penta His antibodies which recognize the his-tagged MSP1D1 protein.
15. Concentrate the elution fractions which contain the nanodiscs with inserted membrane protein using protein concentrators.
16. Freeze the nanodiscs containing membrane proteins at -80 °C in a solution containing 10 % glycerol for future use, or use them directly in the desired experiment.

POPC: Reconstitution of membrane proteins into nanodisc using his-tagged MSP1D1 protein and POPC phospholipids

1. Prepare a membrane protein solution containing your target protein of interest. As a starting point, use 200 µL of the protein solution at a concentration of 2-5 mg/mL. Note: Depending on the target protein, required protein concentrations might be up to 20 mg/mL.
2. Determine the molar concentration of target protein solution, using the molecular weight and extinction coefficient e.g. at 280 nm.
3. Calculate the required amount of MSP protein to be 20 times the molar quantity of the target protein.
4. Calculate the required amount of POPC to be 55 times the molar quantitiy of the MSP protein (or 1,100 times the molar quantity of the target protein). Example: Target protein has a MW of 45,000 Da. Therefore, 500 µg protein in a 200 µL solution corresponds to 11 nmol of target protein in the 1 mL reaction mix. To obtain a 20 fold excess of MSP protein, use 220 nmol of MSP, i.e. 5.57 mg (MW 25.309 g/mol). For a 55 fold excess of phospholipid, use 12.1 µmol or 9.2 mg of POPC (MW 760 g/mol).
5. Resuspend the POPC in 500 µL ND Lipid Buffer II and incubate for 20 min at 37 °C to fully solubilize the phospholipid.
6. Resuspend the MSP protein in 500 µL ND Protein Buffer in a 2 mL microtube. Add the target protein, solubilized in detergent solution. Note: The protein was lyophilized from a solution containing 4 mg/mL protein in 20 mM Tris pH 7.4, 100 mM NaCl, 0.5 mM EDTA.
7. Add the POPC solution to the MSP and membrane protein solution, and incubate the entire mix at 4 °C for 2 h.
8. During the incubation, equilibrate 2.5 g biobeads or similar adsorbant in ND Protein Buffer according to the manufacturer's instructions. Degas the solution by ultrasound to remove any oxygen solubilized in the biobead solution.
9. Add the protein/POPC mix to 750 µL of the biobead solution and incubate at 4 °C on an end over end shaker for 8-12 h.
10. Spin the solution at 10-12,000 x g for 2 min, and transfer the supernatant to a fresh tube. Add 750 µL of fresh equilibrated biobead solution and incubate at 4 °C on an end over end shaker for 8-12 h.
11. Repeat step 10 at least one more time to ensure complete detergent removal.
12. Remove the supernatant, and filter the nanodisc mix through a 0.45 micron filter to remove precipitates that might have formed during incubation.
13. Apply the nanodisc mix on a gel filtration column. Apply the mix in several portions if necessary. Monitor absorbance at 280 nm.
14. Collect fractions of ca. 500 µL size, and analyze the samples by SDS PAGE. MSP proteins have an apparent molecular mass of around 20 kDa. Note: Optionally, analysis of fractions in the western blot can be done e.g. with Penta His antibodies which recognize the his-tagged MSP1D1 protein.
15. Concentrate the elution fractions which contain the nanodiscs with inserted membrane protein using protein concentrators.
16. Freeze the nanodiscs containing membrane proteins at -80 °C in a solution containing 10 %

Application Details

	glycerol for future use, or use them directly in the desired experiment.
Calculation of Results:	SDS-PAGE / Western Blot
Restrictions:	For Research Use only

Handling

Format:	Lyophilized
Storage:	-20 °C