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Nanodisc Assembly Kit MSP2N2_POPC

Product	Catalog No.
Nanodisc Assembly Kit MSP2N2_POPC	**26283**

Kit components	Amount	Packaging
MSP2N2 lyophilized protein	2 mg	Plastic vial, orange cap
Palmitoyl-oleoyl-phosphatidylcholine (POPC)	9 mg	Brown glass vial, white marked cap
Sodium cholate	2x20 mg	Plastic vials, clear cap

Product Description

Nanodiscs were first described by Sligar and coworkers (1, 2). They provide a phospholipid bilayer system held together by membrane scaffold proteins (MSPs). MSPs are truncated forms of apolipoprotein (apo) A-I which wrap around a patch of a lipid bilayer to form a disc-like particle or nanodisc (3). MSPs provide a hydrophobic surface facing the lipids, and a hydrophilic surface at the outside. This setup makes nanodiscs highly soluble in aqueous solutions and allows for the solubilization of membrane proteins in the absence of detergents. These nanobilayer particles are about 7-18 nm in diameter, depending on the mutation variant of MSP used. Most widely employed are MSP1D1-ΔH5, MSP1D1, MSP1E3D1 and MSP2N2, but also other deletion mutants of MSP1D1 are suitable for the generation of nanodiscs (3).
Most commonly used phospholipids are dimyristoyl-glycero-phosphocholine (DMPC) or palmitoyl-oleoyl-phosphatidylcholine (POPC) in combination with sodium cholate.

Cube Biotech offers nanodisc assembly kits that contain the lyophilized proteins and pre-aliquoted amounts of lipids and sodium cholate. See the dedicated protocol for the generation of nanodiscs. They are intended to prepare preassembled nanodiscs that can be added to cell-free expression reactions. During protein synthesis, the nascent membrane protein integrates into the nanodisc. (4).

From 2 mg of MSP protein, this protocol yields about 50 µl nanodiscs in a concentration of about 10-15 mg/ml, corresponding to about 0.5-0.6 mM. We recommend to use an end concentration of 10-100 µM in an *E.coli* cell-free extract, depending on the membrane protein expression rate. This corresponds to 1-10 µl of a nanodisc solution concentrated to 0.5 mM in a total cell-free reaction volume of 50 µl.
With this method, detergents are not required, minimizing possible artifacts. Yields obtained in cell-free expression systems are usually limited to a few micrograms of protein, but offer the possibility to include modifications such as biotinylation or isotope labelling.

Pre-assembled nanodiscs are available from Cube Biotech with different MSP protein: lipid combinations. In addition, Cube Biotech offers wild-type MSP1D1, MSP1E3D1, MSP1D1ΔH5 and MSP2N2 proteins, both as his-tagged and untagged versions. They can be used for the incorporation of proteins that have already been solubilized into detergent micelles.

Nanodiscs are also an important part of our membrane protein service offering. Both empty nanodiscs and recombinant membrane proteins reconstituted into nanodisc are available. Please contact us for details.

Technical details

MSP2N2 protein, lyophilized
Purity: > 90% (SDS-PAGE)
Number of amino acids: 371
Molecular mass: 43,05 kDa
Extinction coefficient (in water) ε_{280}: 36,900 $M^{-1}cm^{-1}$

Palmitoyl-oleoyl-phosphatidylcholine (POPC):
CAS-No. 26853-31-6
Molecular mass: 760.08 Da
Formula: $C_{42}H_{82}NO_8P$

Sodium cholate:
CAS-No. 361-09-1
Molecular mass: 430.57 g/mol
Formula: $C_{24}H_{39}NaO_5$

Shipping & Storage

Shipment Temperature	Ambient temperature
Storage of lyophilized components	-20°C for several months
Storage of reconstituted components	2-8°C for several days

Protein overview and amino acid sequence

Please note: His tag is cleaved off with TEV protease, protease removed by reverse IMAC

MSP2N2 - His
Membrane scaffold protein 2N2
his-tagged

Legend: H: 7xHis, L/L2: Linker / Linker 2 T: *Tev* site, H0.5-H10: Helices 0.5-10, Helices 2-10 are repeated in the MSP2N2 variant.

7x His: GHHHHHHH
Linker: DYDIPTT
Tev site: ENLYFQG
Helix 0.5: STFSKLREQLG
Helix 2: PVTQEFWDNLEKETEGLRQEMS
Helix 3: KDLEEVKAKVQ
Helix 4: PYLDDFQKKWQEEMELYRQKVE
Helix 5: PLRAELQEGARQKLHELQEKLS
Helix 6: PLGEEMRDRARAHVDALRTHLA
Helix 7: PYSDELRQRLAARLEALKENGG
Helix 8: ARLAEYHAKATEHLSTLSEKAK
Helix 9: PALEDLRQGLL
Helix 10: PVLESFKVSFLSALEEYTKKLNTQ
Linker2: GT
Helix 2-II: PVTQEFWDNLEKETEGLRQEMS
Helix 3-II: KDLEEVKAKVQ
Helix 4-II: PYLDDFQKKWQEEMELYRQKVE
Helix 5-II: PLRAELQEGARQKLHELQEKLS
Helix 6-II: PLGEEMRDRARAHVDALRTHLA
Helix 7-II: PYSDELRQRLAARLEALKENGG
Helix 8-II: ARLAEYHAKATEHLSTLSEKAK
Helix 9-II: PALEDLRQGLL
Helix 10-II: PVLESFKVSFLSALEEYTKKLNTQ

Additional Information

For additional nanodisc protocols, please visit our webpage at: www.cube-biotech.com/protocols. For background information on nanodiscs and possible applications please see http://www.cube-biotech.com/background-tips-and-tricks/what-are-nanodiscs.

For protein affinity purification, Cube Biotech offers dedicated agarose resins, magnetic beads and prepacked cartridges. Also available are a range of ultrapure detergents and buffers for extraction and purification of proteins. See www.cube-biotech.com/products for details.

Literature references

1. Bayburt, T.H. et al. Reconstitution and imaging of a membrane protein in a nanometer-size phospholipid bilayer. J. Struct. Biol. (1998), 123(1):37-44

2. Civjan, N.R. et al. Direct solubilization of heterologously expressed membrane proteins by incorporation into nanoscale lipid bilayers. BioTechniques (2003) 35:556-563

3. Hagn, F. et al. Optimized phospholipid bilayer nanodiscs facilitate high-resolution structure determination of membrane proteins. J.Am.Chem. Soc. (2013), 135:1919-1925

4. Proverbio D., et al. Functional properties of cell-free expressed human endothelin A and endothelin B receptors in artifical membrane environments. Biochim.Biophys. Acta (2013), 1828(9):2182-92

5. Roos, C., et al. 2014, High-level Cell-free production of membrane proteins with Nanodiscs. In: Alexandrov, K., and Johnston W.A. (eds) Cell-free protein synthesis: Methods and Protocols. Methods in Molecular Biology, vol. 1118, Springer Science+Business Media.