Cube Biotech
Alfred-Nobel-Str. 10 • 40789 Monheim • Germany
Phone: +49 (0)2173 993730
contact@cube-biotech.com
www.cube-biotech.com

Assembled nanodisc mouse MSP1D1-His_POPC

Product	Tube color	Catalog No.
Nanodisc mouse MSP1D1-His_POPC (50 µl), 500 µM	**clear**	**26713**

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 7-13 nm in diameter, depending on the mutation variant of MSP used. Most widely employed are the MSP1D1, and MSP1E3D1 variants, but also other mutants of MSP1D1 are suitable for the generation of nanodiscs (3).

Most commonly used phospholipids are the eucaryotic dimyristoyl-glycero-phosphocholine (DMPC) and palmitoyl-oleoyl-phosphatidylcholine (POPC) or the procaryotic lipid 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG) in combination with sodium cholate.

For applications where human MSP proteins might affect results (such as immunization of mice), mouse MSP proteins provide a suitable alternative. These MSPs have been produced by aligning the human and mouse MSP sequences in silico, followed by expressing the mouse homologue sequence in *E.coli*.

Cube Biotech offers pre-assembled nanodiscs that can be added to cell-free expression reactions. During protein synthesis, the nascent membrane protein integrates into the nanodisc (4). Typical concentrations of nanodiscs in the cell-free reaction range from 20 to 80 µM. This corresponds to 2-8 µl of a 500 µM nanodisc stock solution 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.

For the incorporation of proteins already solubilized in detergent, mouse MSP1D1-His and MSP1E3-His proteins are available in lyophilized form.

Shipping & Storage

Shipment Temperature	**dry ice**
Storage	**-80°C for several months**

Quality control

To determine efficiency of membrane protein integration into the pre-assembled nanodiscs, the bacterial transporter SugE is expressed in a cell-free lysate as GFP fusion protein. After centrifugation, the percentage of fluorescent protein in the supernatant is measured. Upon addition of 80 µM of nanodisc to the reaction mixture, the nascent protein is integrated and is found in the soluble fraction. Note that no detergent is present in these setups. Absolute expression levels varied from 0.5 mg/ml (MSP1 DMPC) to 1.38 mg/ml (MSP1E3 DMPG).

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 reference

1. Roos, C., Kai, L., Haberstock, S., Proverbio, D., Ghostastider, U., Ma, Y., Filipek, S., Wang, X., Dötsch, V., and Bernhard, F. (2014) High level cell-free production of membrane proteins with nanodiscs. Meth. Mol. Biol. 1118, 109-30
2. Roos, C., Zocher, M., Müller, D., Münch, D., Schneider, T., Sahl, H.G., Scholz, F., Wachtveitl, J., Ma, Y,. Proverbio, D., Henrich, E., Dötsch, V. and Bernhard, F. (2012) Characterization of co-translationally formed nanodisc complexes with small multidrug transporters, proteorhodopsin and with the *E.coli* MraY translocase. Biochim. Biophys. Acta 1818, 3098-106.