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

Assembled nanodisc MSP1E3D1_DMPG
(His tag removed by enzymatic digest)

Product	Catalog No.
Nanodisc MSP1E3D1_DMPG (50 µL), 500 µM	**26365**

Product Description

Nanodiscs were first described by Sligar and coworkers. 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. 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 7-17 nm in diameter, depending on the mutation variant of MSP used. Most widely employed are the standard size MSP1D1 and MSP1E3D1, but also smaller proteins such as MSP1D1ΔH5 (or MSP1D1 dH5) and larger variants such as MSP2N2 are suitable for the generation of nanodiscs.

Most commercially offered MSP proteins carry a his-tag for ease of purification and immobilization. However, if the membrane protein of interest itself is his-tagged, it can be helpful to cleave off the his-tag from the nanodisc scaffold protein. The MSP used for this product has been treated with proteases to remove the his-tag. Remaining his-tagged MSP and the protease itself are removed in a reverse his-tag purification step.

Typically used phospholipids are the eukaryotic lipids dimyristoyl-glycero-phosphocholine (DMPC) and palmitoyl-oleoyl-phosphatidylcholine (POPC) or the prokaryotic lipid 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG) in combination with sodium cholate. 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.

In addition, Cube Biotech offers nanodisc assembly kits for the generation of pre-assembled nanodiscs for use in cell-free systems with a variety of different MSP proteins, in combination with DMPC and POPC. For the incorporation of proteins which are already solubilized in detergent, lyophilized scaffold proteins MSP1D1, MSP1E3, MSP2N2 and MSP1D1ΔH5 (or MSP1D1 dH5) are available.

Protocol

1. Set up cell-free expression reactions according to the manufacturer's instructions. Start with a small scale experiment (50-100 µl) to optimize conditions. Add assembled nanodiscs in concentrations of 5-80 µM, depending on the efficiency of the cell-free reaction. The higher the protein expression efficiency, the more nanodiscs are required to accommodate the nascent protein. When used with Cube Biotech Cell-free Lysates, 20-80 µM should be used, as they provide a high membrane protein synthesis rate. This corresponds to 2-8 µl of a 500 µM nanodisc stock solution in a total cell-free reaction volume of 50 µl.

2. During protein synthesis, the nascent membrane protein integrates into the nanodisc (2). After protein expression (for suitable incubation time refer to the manufacturer's protocol), harvest the reaction by centrifugation (10 min, 20,000 x g) and transfer the supernatant to a fresh tube. The membrane protein/nanodisc complex should be in this fraction. Wash the pellet fraction with PBS.
3. Analyze both the supernatant and pellet fractions by SDS-PAGE to determine integration success rate. Optimize conditions using different nanodisc concentrations and phospholipid-MSP combinations.
4. Once optimal conditions have been determined in small scale, cell-free reactions can be scaled up to volumes of 250-1000 µl.

Shipping & Storage

Shipment Temperature	**dry ice**
Storage	**-80°C for several months** **Avoid repeated freeze-thaw cycles.**

Quality control

Efficient removal of the his-tag from the scaffold protein is determined by Western Blot analysis using an anti-his antibody.
To determine efficiency of the nanodisc assembly reaction, and to ensure homogeneity of the resulting preparation, assembled nanodiscs are applied to a gel filtration chromatography column. Peak position and shape are monitored. Only relevant peak fractions are collected, thereby removing potential reaction by-products such as incompletely assembled membrane scaffold proteins and phospholipids.

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.

Literature references

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.

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