

Assembled nanodisc MSP1D1_POPC Biotinyl PE

Product	Catalog No.
Assembled nanodisc MSP1D1-His_POPC- biotinyl PE (50 µL), 500 µM	26413

Product Description

Membrane scaffold protein	MSP1D1-His
Lipids	1-Palmitoyl-2-oleoyl-SN-glycero-3-phosphocholine (POPC) + 10% 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(biotinyl) (Biotinyl-PE 18:1) headgroup modified lipid
Concentration	500 µM
Quantity	50 µL

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 commonly 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.

For the assembly of this nanodisc product, a mixture of 10% headgroup-biotinylated PE lipid in POPC has been used. The resulting nanodisc-protein-complexes can be used e.g. for immobilization on streptavidin-coated surfaces.

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

Shipping Conditions	dry ice
Storage Conditions	-80°C for several months Avoid repeated freeze-thaw cycles.

Quality control

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., Haberstoch, 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.

Disclaimer: Our products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Nanodiscs are protected by US Patents 7,691,414; 7,662,410; 7,622,437; 7,592,008; 7,575,763; 7,083,958; 7,048,949



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