3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Membrane Scaffold Protein 1E3D1
recombinant, expressed in *E. coli*
buffered aqueous solution

Catalog Number **MSP08**
Storage Temperature –20 °C

Synonyms: MSP1E3D1

Product Description

Nanodisc technology is a widely applicable approach to render membrane proteins soluble in aqueous solutions in a native-like bilayer environment, where the membrane proteins remain stable and active.

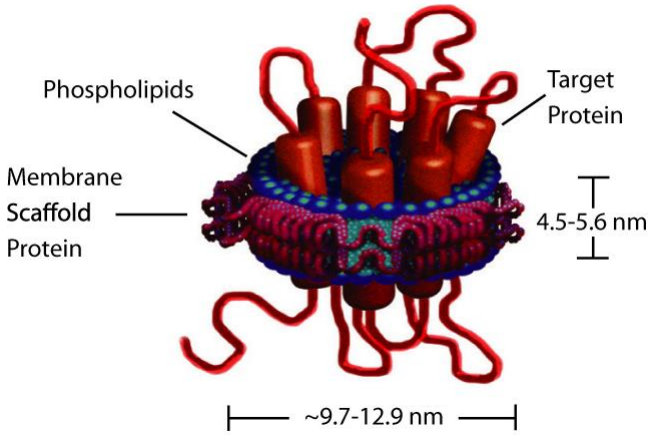
The Nanodisc concept is derived from high density lipoprotein (HDL) particles and their primary protein component, apolipoprotein. The Nanodisc is a non-covalent structure of phospholipid bilayer and membrane scaffold protein (MSP), a genetically engineered protein, which mimics the function of Apolipoprotein A-1 (ApoA-1).[1,2] A soluble Nanodisc assembles as the phospholipid forms a bilayer, which is encircled by two amphipathic MSP molecules that cover the hydrophobic alkyl chains of the bilayer. The length of the MSP controls the size of the Nanodisc structure.[3]

Incorporation of a membrane protein target into a Nanodisc is accomplished by first solubilizing the target protein in detergent, and then mixing with cholate-solubilized phospholipid and MSP. Removal of the detergents results in the assembly of the Nanodisc structure around the target protein (see Figure 1).

The resulting structure renders the protein soluble in a model membrane system of defined phospholipid content and size with a native-like bilayer. This provides stability and functional requirements for the protein target. The size of the Nanodisc, determined by the length of the MSP, allows control of the oligomerization state of the membrane protein.

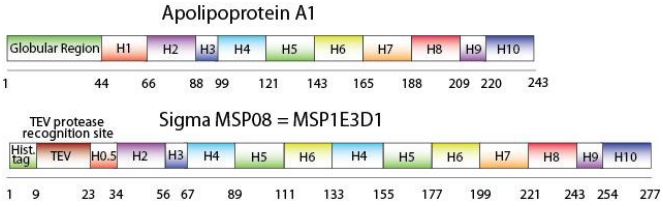
Some examples of membrane-bound proteins incorporated into Nanodiscs are GPCRs,[4] cytochromes P450,[5-7] bacteriorhodopsin,[8] coagulation factors,[9] cholera toxin,[10] and TAR receptor.[11]

Figure 1.
Example of a Nanodisc containing a 7-transmembrane protein



A critical component of Nanodiscs is the membrane scaffold protein (MSP), which forms the encircling amphipathic helical protein belt.[12,13] The sequence similarities of MSPs to Apolipoprotein A-1 are presented in Figure 2.

Figure 2.
Protein Maps of Apolipoprotein A-1 and MSP1E3D1



The first MSP, MSP1, was engineered with its sequence based on the sequence of A-1, but without the globular N-terminal domain of native A-1.[14] The MSP1E3D1 variant of MSP1 differs from MSP1 in the following facets:

- It deletes the first 11 amino acids in the Helix 1 portion (referred to as "H0.5" in Figure 2) of the original MSP1 sequence[3] (which is known separately as MSP1D1). The MSP1D1 protein is an N-terminal histidine-tagged protein with a TEV protease cleavage site between the histidine-tag and the protein sequence.
- It repeats the Helix 4 (H4), Helix 5 (H5) and Helix 6 (H6) sequences of the original MSP1 sequence between the parent Helix 6 (H6) and Helix 7 (H7) segments of MSP1D1.[15]

MSP1E3D1 yields Nanodiscs on the order of ~12.1 nm.[16] The thickness of a Nanodisc is dependent on the type of phospholipid incorporated (typically 4.6–5.6 nm).[3]

This product is supplied as a buffered aqueous solution, at a protein concentration of ~5 mg/mL, and containing 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.5 mM EDTA, 5 mM sodium cholate, and 0.1% sodium azide.

MSP1E3D1 sequence:[17]
GHHHHHHHDYDIPTTENLYFQGSTFSKLREQLGPVT
QEFWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDF
QKKWQEEMELYRQKVEPLRAELQEGARQKLHELQE
KLSPLGEEMRDRARAHVDALRTHLAPYLDDFQKKWQ
EEMELYRQKVEPLRAELQEGARQKLHELQEKLSPLG
EEMRDRARAHVDALRTHLAPYSDELRQRLAARLEAL
KENGGARLAEYHAKATEHLSTLSEKAKPALEDLRQGL
LPVLESFKVSFLSALEEYTKKLNTQ

Molecular mass: 32,599.6 Da (based on sequence)
Molecular mass of histidine-tag cleaved variant: 29,981.9 (based on sequence)

The MSP1E3D1 protein concentration can be determined spectrophotometrically using the following extinction coefficients:

MSP1E3D1, ε_{280} = 29,910 M^{-1} cm^{-1}
Histidine-tag cleaved variant, ε_{280} = 26,930 M^{-1} cm^{-1}
(Solvent: 20 mM Tris, pH 7.4, with 0.1 M NaCl, 0.5 mM EDTA, and 0.01% NaN_3)

Reagents Required, but Not Provided

- Sodium Cholate (Catalog No. C6445)
- Amberlite® XAD®-2 (Catalog No. 20275)
- Phospholipid(s)
  - 2-Oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (POPC, Catalog No. P3017)
  - 1,2-Dipalmitoyl-*rac*-glycero-3-phosphocholine (DPPC, Catalog No. P5911)
  - 1,2-Dimyristoyl-*rac*-glycero-3-phosphocholine (DMPC, Catalog No. P7930)
- Triton™ X-100 (optional, Catalog No. X100)
- Superdex® 200 (Catalog No. S6782)
  or
  Superdex 200 Increase 10/300 GL column (Catalog No. GE28-9909-44)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store this product at –20 °C.

Procedure
The successful assembly of Nanodiscs is highly dependent on the properties of the target protein. The choice of the types and ratios of MSPs, phospholipids, and detergents might require trials and titrations to optimize disc assembly. **A thorough review of the literature is essential for this aspect of Nanodisc construction.**[3,12,13,14]

Successful incorporation of the target membrane protein into Nanodiscs most likely will require optimization of the following important parameters:

- Choice and concentration of secondary detergent for preparation of target membrane protein
- Choice and preparation of phospholipid
- Temperature of reconstitution
- Lipid to MSP ratio
- MSP to target protein ratio

References

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17. Supplier information.

For a further and extensive list of citations and protocols, visit the website of Professor Stephen Sligar, University of Illinois:
http://sligarlab.life.uiuc.edu/nanodisc.html

Nanodisc technology, and many of its uses, are covered by the following patents held by the University of Illinois:

- 7,691,414: Membrane scaffold proteins
- 7,662,410: Membrane scaffold proteins and embedded membrane proteins
- 7,622,437: Tissue factor compositions and methods
- 7,592,008: Membrane scaffold proteins
- 7,575,763: Membrane scaffold proteins and tethered membrane proteins
- 7,083,958: Membrane scaffold proteins
- 7,048,949: Membrane scaffold proteins

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