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Product Information

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

Catalog Number **M7074**
Storage Temperature –20 °C

Synonym: 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 they remain stable and active.

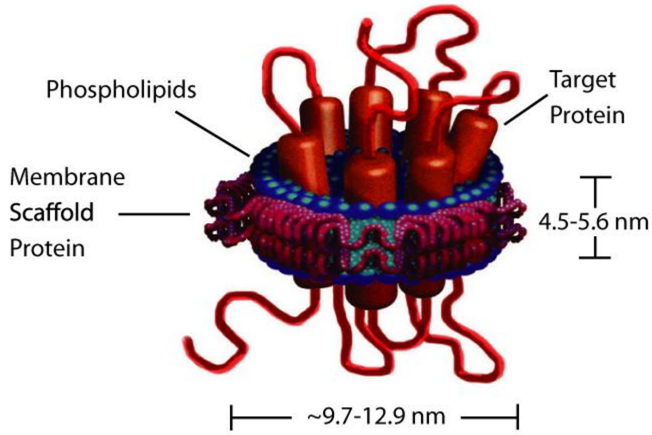
The Nanodisc concept is derived from high density lipoprotein (HDL) particles and their primary protein component, apolipoprotein. The Nanodisc is a non-covalent structure of phospholipid bilayer and membrane scaffold protein (MSP), a genetically engineered protein, which mimics the function of Apolipoprotein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

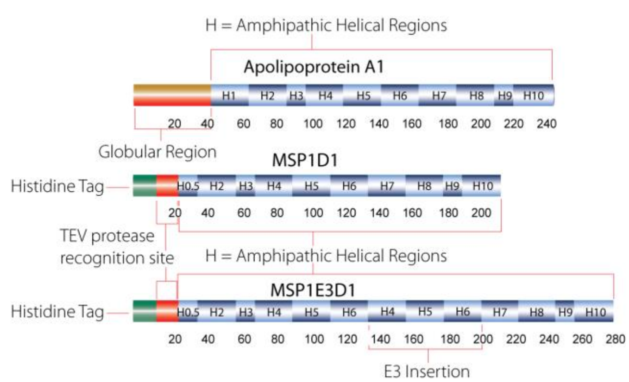
Some examples of membrane-bound proteins incorporated into Nanodiscs are GPCRs,[4] cytochromes P450,[5-7] bacteriorhodopsin,[8] coagulation factors,[9] cholera toxin,[10] and TAR receptor.[11]

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 MSPs



The first MSP, MSP1, was engineered with its sequence based on the sequence of Apolipoprotein A-1, but without the globular N-terminal domain of native A-1.[14] The Membrane Scaffold Protein 1E3D1 (MSP1E3D1) variant of MSP1 contains an additional 3 helix sequences, and may be used to generate larger Nanodiscs compared to those derived from MSP1.[3]

MSP1E3D1 yields Nanodiscs of ~12.9 nm. The thickness of a Nanodisc is dependent on the type of phospholipid incorporated (typically 4.6–5.6 nm).[3]

MSP1E3D1 is supplied as a lyophilized powder stabilized with Tris-HCl, and containing NaCl and EDTA. It is supplied as an N-terminal histidine-tagged protein with a TEV protease cleavage site between the histidine-tag and the protein sequence.

MSP1E3D1 sequence:[15]
GHHHHHHHDYDIPTTENLYFQGSTFSKLREQLGPVT
QEFWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDF
QKKWQEEMELYRQKVEPLRAELQEGARQKLHELQE
KLSPLGEEMRDRARAHVDALRTHLAPYLDDFQKKWQ
EEMELYRQKVEPLRAELQEGARQKLHELQEKLSPLG
EEMRDRARAHVDALRTHLAPYSDELRQRLAARLEAL
KENGGARLAEYHAKATEHLSTLSEKAKPALEDLRQGL
LPVLESFKVSFLSALEEYTKKLNTQ

Molecular mass: 32,599.98 Da (based on sequence)

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

MSP1E3D1, ε_{280} = 29,400 M^{-1} cm^{-1}
Histidine-tag cleaved variant, ε_{280} = 26,600 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.

Preparation Instructions
MSP1E3D1 Stock Solution: Reconstitution of MSP1E3D1 at 5 mg of protein per ml of sterile deionized water results in a protein solution containing 20 mM Tris, pH 7.4, with 0.1 M NaCl and 0.5 mM EDTA.

Storage/Stability
The product ships at ambient temperature and storage at –20 °C is recommended. When stored at –20 °C, the protein remains functional for at least 1 year.[16]

The MSP1E3D1 Stock Solution can be stored at 2–8 °C for up to several days. Recommended long-term storage is in frozen aliquots at –20 °C.[16]

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

1. Brouillette, C.G. *et al*., *Biochim. Biophys. Acta*, **1531(1-2)**, 4-46 (2001).
2. Jonas, A., *Methods Enzymol*., **128**, 553-582 (1986).
3. Denisov, I.G. *et al*., *J. Am. Chem. Soc*., **126(11)**, 3477–3487 (2004).
4. Bayburt, T.H. *et al*., *J. Biol. Chem*., **282(20)**, 14875-14881 (2007).
5. Denisov, I.G. *et al*., *J. Biol. Chem.*, **282(10)**, 7066-7076 (2007).
6. Grinkova, Y.V. *et al*., *Biochem. Biophys. Res. Commun*., **372(2)**, 379-382 (2008).
7. Denisov, I.G. *et al*., *Biochim. Biophys. Acta*, **1814(1)**, 223-229 (2011).
8. Bayburt, T.H. *et al*., *Arch. Biochem. Biophys*., **450(2)**, 215-222 (2006).
9. Shaw, A.W. *et al*., *J. Biol. Chem.*, **282(9)**, 6556-6563 (2007).
10. Borch, J. *et al.*, *Anal. Chem.*, **80(16)**, 6245-6252 (2008).
11. Boldog, T. *et al.*, *Proc. Nat. Acad. Sci. USA*, **103(31)**, 11509-11514 (2006).
12. Nath, A. *et al.*, *Biochemistry*, **46(8)**, 2059-2069 (2007).
13. Bayburt, T.H., and Sligar, S.G., *FEBS Lett*., **584(9)**,1721-1727 (2010).
14. Bayburt, T.H. *et al., Nano Letters,* **2(8)**, 853-856 (2002).
15. Sligar Lab Website: sligarlab.life.uiuc.edu/nanodisc/sequences.html
16. Supplier data.

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

TD,RC,RBG,GCY,MAM 10/16-1