

MSP Starter Kit for In Vitro Protein Synthesis, human

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Description

The MSP Starter Kit for In Vitro Protein Synthesis is ready to use for cell-free protein synthesis: it contains 8 different pre-assembled MSP nanodiscs, which can be added directly to the expression reaction so the membrane protein inserts co-translationally into a membrane with predefined lipid composition, no detergents or post-synthesis reconstitution needed. Two scaffold sizes (MSP1D1, MSP1E3D1) × four lipid compositions (DOPG; DOPG/Cardiolipin; POPC/POPS; POPC/POPS/Cholesterol) allow quick identification of the best-performing condition, manually or on a liquid handler. The His-tagged version (cat. No. 26702) enables fast affinity purification, and matching scale-up vials carry the winning condition forward to µg-to-mg quantities of purified, functional receptor.

The composition of the kit is 8 different pre-assembled MSP nanodiscs.

MSP	Lipid composition
MSP1D1	DOPG
	DOPG/Cardiolipin
	POPC/POPS
	POPC/POPS/Cholesterol
MSP1E3D1	DOPG
	DOPG/Cardiolipin
	POPC/POPS
	POPC/POPS/Cholesterol

1. Required material & recommendations

Cell-free expression reaction mix

The MSP Starter-Kit for In Vitro Protein Synthesis, 26712

The MSP Starter-Kit for In Vitro Protein Synthesis (-His), 26702

2. Protocol

2.1. Preparation of In vitro protein synthesis

- 2.1.1.** Set up the cell-free expression reaction according to the manufacturer's instructions. A total volume of 50 µl is sufficient.
- 2.1.2.** Add 7.5 µl of assembled MSP nanodisc (final concentration of 5–75 µM), depending on the efficiency of the cell-free reaction. The higher the protein expression efficiency, the more MSP nanodisc is required to accommodate the nascent protein.
- 2.1.3.** Protein synthesis can take time; check the incubation time in the manufacturer's instructions. During this time, the nascent membrane protein integrates into the MSP nanodisc.
- 2.1.4.** After protein expression, harvest the reaction by centrifugation for 10 min at 20,000 x g, and transfer the supernatant to a fresh tube.
- 2.1.5.** The membrane protein/MSP nanodisc complex should be in this fraction.
- 2.1.6.** Wash the pellet fraction with PBS.
- 2.1.7.** Analyze both the supernatant and pellet fractions by SDS-PAGE to determine integration success rate. Optimize conditions using different MSP nanodisc concentrations and phospholipid-MSP combinations.
- 2.1.8.** Once optimal conditions have been determined at small scale, cell-free reactions can be scaled up to volumes of 250–1000 µl.

3. Troubleshooting

Cell-free expression	
No or low protein expression	Check DNA stock quality (sequencing, fresh DNA prep, purity measurement) or optimize DNA concentration by screening (recommended as first step in any cell-free project).
General process failure	Include a detergent control (e.g. Brij) to verify that the overall cell-free process is functioning correctly.
Inconsistent or poor results	Prepare fresh stocks, recalibrate pipettes, increase incubation time (cell-free synthesis relies heavily on precision).
Protein detected in pellet instead of supernatant	Perform Western Blot on pellet fraction – if positive: protein expression worked, but solubilization or stabilization into MSP nanodiscs failed; optimize the MSP:protein ratio.
Protein not solubilized / not stable in MSP	Review MSP lipid composition and consider testing alternative lipid mixtures.