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-tag 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
MSPE3D1	DOPG
	DOPG/Cardiolipin
	POPC/POPS
	POPC/POPS/Cholesterol

1. Required material & recommendations

Cell-free expression reaction mix
The MSP Starter-Kit

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.