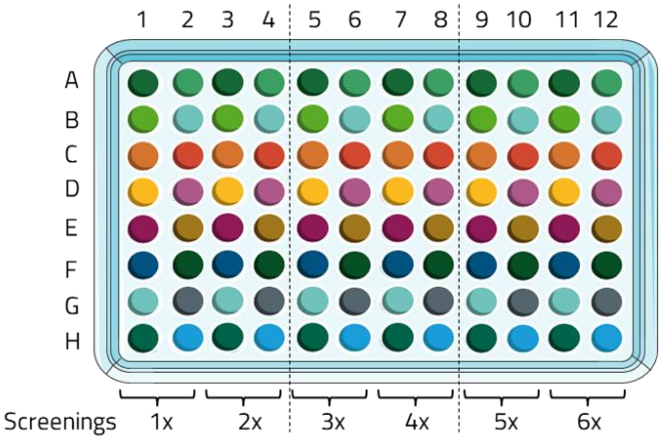
Membrane protein screening and purification using NativeMP™ Screening & Strep-Tactin®XT Purification plates with KingFisher™ Flex

Automated membrane protein screening & purification with KingFisher™ Flex

1. Description

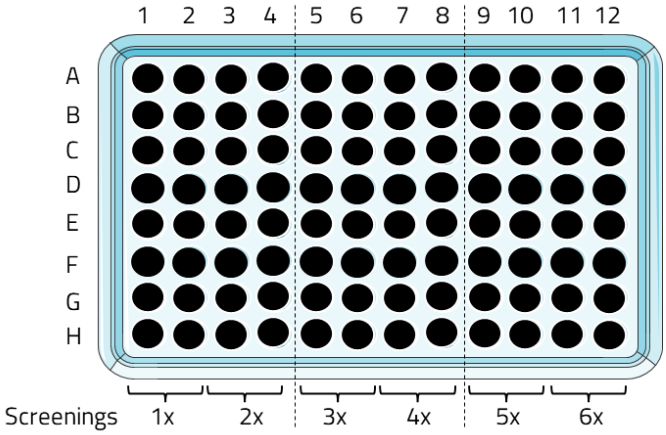
The NativeMP™ Screening Plate is a ready-to-use solution for automated membrane protein solubilization screening. It enables rapid identification of the most effective synthetic copolymer, selected from sixteen variants, for subsequent scale-up of membrane protein production. The plate contains 2×, 4×, or 6× of the same copolymer (25 mg per well) in lyophilized format, allowing up to six parallel screenings.



Cat. No.	90302	90304	90306
Copolymer	2x Screenings	4x Screenings	6x Screenings
Cubipol	A1, A3	A1, A3, A5, A7	A1, A3, A5, A7, A9, A11
Cubipol Glycerol	B1, B3	B1, B3, B5, B7	B1, B3, B5, B7, B9, B11
Sulfo-Cubipol	C1, C3	C1, C3, C5, C7	C1, C3, C5, C7, C9, C11
Sulfo-Cubipol Lite	D1, D3	D1, D3, D5, D7	D1, D3, D5, D7, D9, D11
Sulfo-Cubipol Medium	E1, E3	E1, E3, E5, E7	E1, E3, E5, E7, E9, E11
Glyco-Cubipol	F1, F3	F1, F3, F5, F7	F1, F3, F5, F7, F9, F11
Cubipol PEG	G1, G3	G1, G3, G5, G7	G1, G3, G5, G7, G9, G11

Cubipol Amine	H1, H3	H1, H3, H5, H7	H1, H3, H5, H7, H9, H11
Ultrasolute™ Amphipol 17	A2, A4	A2, A4, A6, A8	A2, A4, A6, A8, A10, A12
Ultrasolute™ Amphipol 18	B2, B4	B2, B4, B6, B8	B2, B4, B6, B8, B10, B12
AASTY 6-50	C2, C4	C2, C4, C6, C8	C2, C4, C6, C8, C10, C12
AASTY 11-50	D2, D4	D2, D4, D6, D8	D2, D4, D6, D8, D10, D12
SMA200	E2, E4	E2, E4, E6, E8	E2, E4, E6, E8, E10, E12
Sulfo-SMA	F2, F4	F2, F4, F6, F8	F2, F4, F6, F8, F10, F12
DIBMA 10	G2, G4	G2, G4, G6, G8	G2, G4, G6, G8, G10, G12
Sulfo-DIBMA	H2, H4	H2, H4, H6, H8	H2, H4, H6, H8, H10, H12

The NativeMP™ Strep-Tactin®XT Purification Plate is 96 deep-well plates designed for high-throughput purification of membrane proteins fused to Strep-tag®II or Twin-Strep-tag® using KingFisher instruments. These plates contain dehydrated MagStrep® Strep-Tactin®XT beads (30 µl per well), respectively, all characterized by high binding specificity and affinity.



Cat. No.	90302	90304	90306
NativeMP™ Strep-Tactin®XT Purification plate	2x Screenings	4x Screenings	6x Screenings
MagStrep® Strep-Tactin®XT beads	A1-H4	A1-H8	A1-H12

For optimal results, NativeMP™ Screening Plates and NativeMP™ Strep-Tactin®XT Purification Plates should be used in combination. Together with the appropriate MagBead selection, this integrated approach enables identification of the best solubilization and stabilization conditions for your membrane protein of interest.

2. Required material & recommendations

NativeMP™ Screening Plate, 96 deep-well plate (KingFisher™) buffers/solutions		Cat. No.	Quantity
NativeMP™ Screening plate	25 mg copolymers per well (16 different copolymers per screen)	90302 90304 90306	2x screenings 4x screenings 6x screenings

NativeMP™ Strep-Tactin®XT Purification Plate, 96 deep-well plate (KingFisher™) buffers / solutions		Cat. No.	Quantity
NativeMP™ Strep-Tactin®XT Purification plate	30 µL dehydrated magnetic beads per well	90422 90424 90426	2x screenings 4x screenings 6x screenings
Protein buffer	150 mM NaCl, 20 mM HEPES, pH 7.5, 1x protease inhibitor mix		
Wash buffer	150 mM NaCl, 20 mM HEPES, pH 7.5		
Elution buffer	150 mM NaCl, 20 mM HEPES, pH 7.5, 50 mM biotin	2-1016-002 Biotin peptide	

All components required for setting up the KingFisher™ Flex and subsequent membrane protein isolation are listed in the table below.

Equipment for KingFisher™ Flex Purification System with 96 Deep-Well Head		Cat. No.	Quantity
KingFisher™ Flex 96 heating block	Thermo Scientific™	24075430	1
KingFisher™ Flex 96 Deep Well head	Thermo Scientific™	24074430	1
KingFisher™ 96-tip Comb, for deep-well magnets, 10x10 pcs/box (for flex and Presto)	Thermo Scientific™	97002534	1
KingFisher™ 96 deep-well plate, v-bottom, polypropylene (for Duo Prime, Flex and Presto)	Thermo Scientific™	95040450	1

3. Protocol

3.1. Preparation of the cell lysate

3.1.1. Add protease inhibitors to protein buffer and adjust pH value.

3.1.2. Weight cell pellet and resuspend it in protein buffer with protease inhibitors. Approximately 5 mL of protein buffer with protease inhibitors is required per 1 gram of pellet.

3.1.3. Lyse cells by sonication, French press or other procedures of your choice and pool cell lysates afterwards if necessary.

3.1.4. Centrifuge cell lysate at 9,000 x g for 45 minutes at 15 °C.

3.1.5. Collect supernatant and discard cell debris.

3.1.6. Take a small sample of the supernatant (e.g., 5 µL) and dilute the sample 1/100 in protein buffer. For Blank, dilute protein buffer with protein inhibitors in the same way.

3.1.7. Measure the absorbance of the diluted sample at 280 nm and calculate the absorbance of the supernatant. The absorbance value of the undiluted sample should not exceed 150 AU. If it does, dilute the supernatant. Otherwise, the polymer may not dissolve completely and the liquid viscosity may be too high.

3.2. Equilibration of the MagBeads well plate with KingFisher™ Flex

Download the program for equilibration of MagBeads with KingFisher™ Flex here.

3.2.1. Add 1 mL of wash buffer to each well containing MagBeads.

3.2.2. Prepare three additional equilibration plates containing 1 mL protein buffer in the same layout as the NativeMP™ Strep-Tactin®XT purification plate from Cube Biotech.

3.2.3. Add a Tip comb to a 96 deep well plate.

3.2.4. Load all prepared plates into the KingFisher™ Flex as listed below (or shown on its display). Press start to load next plate.

Position	Plate
5	KingFisher™ 96-tip Comb
4	Equilibration plate 3 with 1 mL Wash buffer
3	Equilibration plate 2 with 1 mL Wash buffer
2	Equilibration plate 1 with 1 mL Wash buffer
1	NativeMP™ Strep-Tactin®XT purification plate

3.2.5. Resuspend the MagBeads by mixing for 10 min at “fast” speed.

3.2.6. Transfer the MagBeads in equilibration plate 1 and incubate 5 min at “medium” speed. Repeat this step with equilibration plate 2.

3.2.7. Store the MagBeads until use in equilibration plate 3.

3.3. Preparation of the wash and elution plates

3.3.1. Prepare three wash plates by adding 1mL wash buffer to the wells corresponding to your plate layout.

3.3.2. Prepare one elution plate by adding at least 50 µL elution buffer to the wells corresponding to your plate layout.

3.4. Protein purification with KingFisher™ Flex

All subsequent steps are performed automatically by KingFisher™ Flex. Download the program here.

3.4.1. Pipette 1 mL of the clarified supernatant directly into the wells of a NativeMP™ screening plate from Cube Biotech containing the dried copolymers (Please add the cell lysate to the copolymers shortly before the start of the KingFisher™ Flex).

3.4.2. Load all the plates prepared as described above in the following order into the KingFisher™ Flex (alternative as shown on its display (press start to load next plate)).

Position	Plate
7	KingFisher™ 96-tip Comb
6	Elution plate with 50 µL Elution buffer
5	Wash plate 3 with 1 mL Wash buffer
4	Wash plate 2 with 1 mL Wash buffer
3	Wash plate 1 with 1 mL Wash buffer
2	Equilibration plate 3 from step 3.2.7
1	NativeMP™ screening plate

3.4.3. Set the temperature to the most suitable value for the target protein (e.g. 22°C).

3.4.5. Solubilize 5 min at "fast" speed.

3.4.6. Transfer the magnetic beads from the MagBeads plate to the copolymer plate and incubate 5 min at "fast" speed without heating.

3.4.7. Wash the MagBeads for 5 min in wash plate 1 at "fast" speed without heating. Repeat this step with wash plate 2 and 3.

3.4.8. Transfer the MagBeads to the elution plate and incubate 15 min at "fast" speed without heating.

3.4.9. Transfer the MagBeads to Equilibration plate 3. The elution plate contains purified membrane protein.

3.5. Analysis	
3.5.1.	Optional: Centrifuge the elution plate at 1.000 x g for 2min with the plate rotor, to pool the elutions at the bottom of the wells.
3.5.2.	Optional: Pipette the elutions from the plate to Eppis and centrifuge at 50,000 × g for 10 min to get rid of any possible aggregation or leftover MagBeads.
3.5.3.	Take ~10 µL for DLS measurement e.g. Nanotemper Prometheus Panta and 25 µL for SDS PAGE and Western Blot.

4. Troubleshooting

Handling of Native MP™ screening & Strep-Tactin®XT purification plates and KingFisher™ Flex	
Insufficient polymer solubilization	Extend the solubilization time and/or increase temperature stepwise (e.g., RT, 30 °C, 37 °C, 42 °C). Otherwise, dilute cell lysate with protein buffer according to absorbance at 280 nm.
Magnetic beads can't be transferred	Cell suspension is too viscous. Dilute cell lysate with protein buffer according to absorbance at 280 nm.
After elution, some magnetic beads are visible at the well wall near the well top.	Increase MagBeads collection time.
Solubilization efficiency & yield	
No visible solubilization/ solution remains turbid	Increase temperature stepwise (e.g., RT, 30 °C, 37 °C, 42 °C) and extend the solubilization time; higher temperature accelerates membrane insertion and nanodisc formation. Depending on cell type, some form of turbidity after solubilization is normal. Check if protein eluates do contain the target protein; if not, proceed with the suggestions.
Very low membrane protein yield	Validate that expression of target protein is high enough. Validate that the right resin for your specific tag was used.

Sample clears, but target protein is not detected	Verify expression level and correct membrane insertion prior to copolymer use; copolymers cannot rescue non-inserted or mislocalized proteins.
Protein stability, function & homogeneity	
Strong aggregation observed by SEC or DLS	Screen for best incubation temperature, time and copolymer concentration; buffer composition can promote nanodisc stacking or aggregation. Make sure signal to noise ratio is sufficient – a low target concentration often results in poor outcomes.
Loss of protein, no ligand binding or loss of activity	Adjust the pH of all buffers according to the requirements of the membrane protein of interest. Lower solubilization temperature and shorten incubation; functional loss often originates from target protein disintegration rather than copolymer chemistry.
Functional protein but poor structural homogeneity	Screen alternative copolymers with different backbone chemistry, side-chain modifications, and charge density; copolymer-lipid interactions strongly influence nanodisc uniformity.
Background, purity & sample quality	
High background in affinity purification	Make sure viscosity is not too high and lysate clears up during solubilization. Add DNAse to reduce viscosity and nonspecific binding.
Sample becomes highly viscous	Add DNAse/Benzonase before copolymer addition; copolymers do not remove nucleic acids and viscosity reduces effective solubilization.
Protein degrades during solubilization	Strengthen protease inhibitor cocktail, reduce processing temperature, and shorten handling time; copolymers do not inactivate proteases.
Plate handling & appearance	
Copolymer solution has a yellow appearance	This is normal and does not affect product efficiency.
White crystals are visible on the magnetic beads film	White crystals may sometimes appear on the magnetic beads film due to the conservation process, which is not a quality defect.
Affinity purification & tag compatibility	

Inefficient binding to affinity resin (Strep-/Twin-Strep-tag)	Verify copolymer compatibility and tag accessibility; biotinylated copolymers cannot be used with streptavidin-based resins (Strep-Tactin®XT). Change tag position or tag if needed.
Reproducibility, scale-up & workflow choice	
Poor reproducibility between experiments	Control expression, buffers, incubation times, temperature and elution conditions strictly.
Different copolymers work in screening but fail at larger scale	Re-optimize copolymer-to-membrane ratio, buffer, and resin ratios after scaling; parameters do not scale linearly with volume.
Inconsistent results between expression systems	Repeat NativeMP™ screening and purification in the final expression host; lipid composition differs strongly between mammalian, insect, yeast, and bacterial membranes.
Downstream assay incompatible with selected copolymer	Select copolymer based on final application, not solubilization efficiency alone; downstream compatibility must be part of screening.
Optimization becomes iterative and slow	Apply the rule: screen first, optimize later; copolymer identity must be fixed before adjusting buffer, temperature, or ratios.