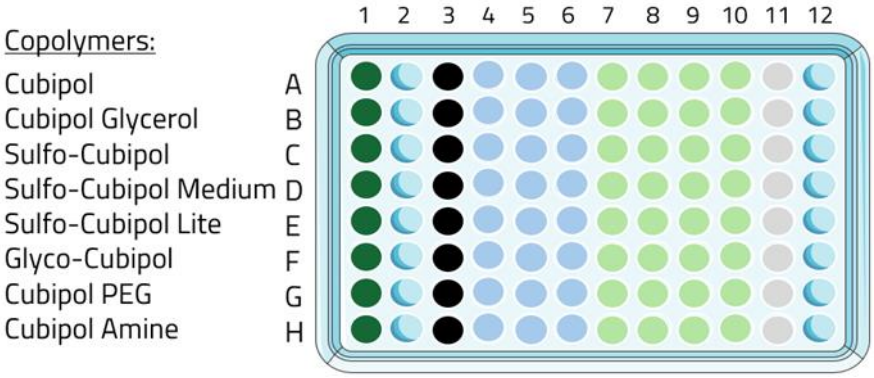
Membrane protein isolation using PlateX MP™ with ASSIST PLUS

Automated NativeMP™ copolymer screening and protein purification within one plate

1. Description

Cube Biotech's PlateX MP™ plates offer a simple solution for solubilization, stabilization, and purification of membrane proteins, enabling small-scale isolation of native and functional membrane proteins. The plate contains eight Cubipols from the NativeMP™ copolymer solution to determine the best stabilizing copolymer in a low-effort screening process (Figure 1). In addition, dehydrated magnetic beads for subsequent protein purification are supplied with the appropriate lyophilized buffers for equilibration, washing, and elution. Only ddH_2O and cell lysate containing the target protein are additionally required. Three plate variants are available specifically for the INTEGRA ASSIST PLUS: either with magnetic beads for Rho1D4-, Strep- or DYKDDDDK (FLAG)-tagged proteins. All three variants are ideally suited for efficient and native purification of membrane proteins.



Column	Content
1	Copolymers as indicated (45 mg/well)
2	Empty column for sample application (1800 µL/well)
3	Dehydrated magnetic beads specific for Rho1D4-, Strep- or FLAG-tagged proteins (30 µL pure beads/well)
4-6	Lyophilized equilibration buffer (150 mM NaCl, 20 mM HEPES, pH 7.5; 950 µL/well)
7-10	Lyophilized wash buffer (150 mM NaCl, 20 mM HEPES, pH 7.5; 950 µL/well)
11	Lyophilized elution buffer (150 mM NaCl, 20 mM HEPES, pH 7.5 and 2 mg/mL Rho peptide or 50 mM biotin or 0.25 mg/mL FLAG peptide depending on the bead specificity for Rho1D4-, Strep- or FLAG-tagged proteins; 250 µL/well)
12	Empty column

Figure 1: Schematic representation of PlateX MP™ plate layout.

2. Required material & recommendations

The protocol is intended for the following PlateX MP™ plates:

Product	Cat. No
PlateX MP™ Rho1D4 MagBeads, 96 deep-well plate (INTEGRA)	90660
PlateX MP™ Anti-DYKDDDDK MagBeads, 96 deep-well plate (INTEGRA)	90760
PlateX MP™ Strep-Tactin®XT MagBeads, 96 deep-well plate (INTEGRA)	90860

All components required for setting up the ASSIST PLUS and subsequent membrane protein isolation are listed in the table below.

Equipment for ASSIST PLUS set up		Cat. No.	Quantity
8-channel VOYAGER pipette with adjustable tip spacing, 50-1250µL	INTEGRA	4724	1
1250 µL Standard pipette tips	INTEGRA	6445	96 tips
100 mL Reservoir Base	INTEGRA	4305	1
Tube Rack	INTEGRA	4540	1
Adapter and magnet for 2.2 ml deep well plate for MAG module	INTEGRA	4907	1
MAG module	INTEGRA	4900	1
Tip Waste Bin	INTEGRA	4573	1
1.5 mL reaction tubes			8

The composition of the recommended protein buffer and the necessary protease inhibitors for cell lysis, as well as the required amount of ddH_2O for solubilizing the dried buffers in the PlateX MP™ plate, are listed in the table below.

Buffers/solutions		Storage until use	Quantity
Protein buffer	150 mM NaCl, 20 mM HEPES pH 7.5	Room temperature	Approximately 5 mL protein buffer per 1 g pellet required.
Protease inhibitor stock solutions	0.01 mM Leupeptin dissvoled in ethanol	-20 °C	Add 100 µL per 100 mL protein buffer.
	0.01 mM E-64 dissolved in ddH_2O	-20 °C	Add 100 µL per 100 mL protein buffer.
	0.1 mM PMSF dissolved in isopropanol	-20 °C	Add 100 µL per 100 mL protein buffer.
	1 µM Pepstatin A dissolved in ethanol:acetic acid (9:1)	-20 °C	Add 100 µL per 100 mL protein buffer.
	0.25 mM Phenanthroline dissolved in ethanol	-20 °C	Add 400 µL per 100 mL protein buffer.
ddH_2O		Room temperature	100 ml

3. Protocol

3.1. Preparation of the cell lysate

3.1.1. Add protease inhibitors (PI) 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.

3.1.8. Pipette 1.8 ml of the prepared cell lysate supernatant in each well of PlateX MP™ column 2 (Figure 1).

3.2. Set up ASSIST PLUS

3.2.1. Install the VOYAGER pipette by attaching to the designated apparatus and connecting to ASSIST PLUS.

3.2.2. Open pipette box with 96 x 1250 µL INTEGRA Standard pipette tips and place into its position under the pipette (first deck position). Make sure the open lid does not interfere with the robotic arm.

3.2.3. Fill 100 ml ddH_2O in the INTEGRA Reservoir Base and place it in the second deck position.

3.2.4. Install INTEGRA Tube Rack in third deck position and insert 8 x 1.5 mL reaction tubes into the last column of the Tube Rack.

3.2.5. Install INTEGRA MAG modul, Adapter and magnet for 2.2 mL deep well plate in the fourth deck position.

3.2.6. Place PlateX MP™ on top of the MAG modul.

3.2.7. Install INTEGRA Tip Waste Bin into the fifth deck position. Add waste container into the box. Make sure the laser is not blocked and the reflective tape is in good shape.

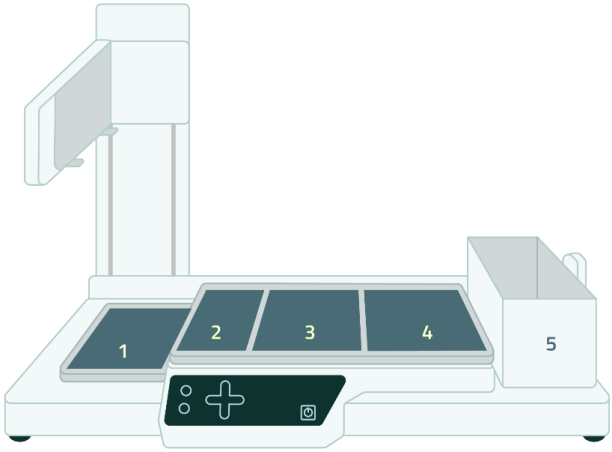


Figure 2: Schematic representation of the INTEGRA ASSIST PLUS and the deck positions (1-5) within the liquid handler.

3.3. Solubilization, stabilization and purification of membrane proteins

All subsequent steps are performed automatically by ASSIST PLUS. Download the program here.

3.3.1. Lyophilized **equilibration and wash buffer** (column 4 to 10) are resuspended with **950 µL ddH_2O**.

3.3.2. Lyophilized **elution buffer** (column 11) is resuspended with **250 µL ddH_2O**.

3.3.3. **Copolymers** are dissolved with cell lysate. For that, transfer **900 µL cell lysate** from column 2 in column 1. Cell lysate and copolymer are **mixed for 250 cycles**. **Transfer remaining 900 µL cell lysate** in column 1. Solution is **mixed for 10 min**.

3.3.4. **Magnetic beads** (column 3) are resuspended and equilibrated with **950 µL equilibration buffer**. Equilibration buffer transfer is started from column 4. Magnetic beads are **mixed** by pipetting up and down for **10 min for the first cycle** and **4 min for the following cycles**. Magnetic beads are separated from buffer by activating the magnet. Supernatant is removed and returned to the initial column. Then the magnet is deactivated.

3.3.5. Step 3.3.4. is repeated **twice**.

3.3.6. **Cell lysate-copolymer mixture** (column 1) is **transferred to magnetic beads** (column 3) by pipetting **twice 900 µL** to capture the stabilized target protein. Mixing occurs for 10 min. Magnetic beads are separated from buffer by activating the magnet. Supernatant is returned to column 1 by pipetting twice 900 µL (flow-through fraction) and then the magnet is deactivated.

3.3.7. **Magnetic beads** (column 3) are washed with **950 µL washing buffer**. Washing buffer transfer is started from column 7. Magnetic beads are **mixed** by pipetting up and down for **2 min**. Magnetic beads are separated from buffer by activating the magnet. Supernatant is removed and returned to the initial column. Then the magnet is deactivated.

3.3.8. Step 3.3.7. is repeated **three times**. During last washing step supernatant is removed in two steps in order to bring magnetic beads closer to the well bottom.

3.3.9. Target protein from **magnetic beads** (column 3) is eluted with **50 µL elution buffer** (column 11). Magnetic beads are mixed by pipetting up and down for 5 min. Magnetic beads are separated from buffer by activating the magnet. Eluate is transferred to column 12 and then the magnet is deactivated.

3.3.10. Step 3.3.9. is repeated **once**.

3.3.11. Magnet is activated and eluate is transferred to an 1.5 mL reaction tube for later use. Afterwards magnet is deactivated.

4. Troubleshooting

Handling of PlateX MP™ and ASSIST PLUS	
Insufficient polymer solubilization	Increase the mix cycle number via variable (mix_cycle_copolymer1). Otherwise, dilute cell lysate with protein buffer according to absorbance at 280 nm.
Clogging during solubilization (copolymer/lysate mixing)	Can occur temporarily, but tips should be cleared in the process. If clogging persists, dilute cell lysate more next time.
Foam in copolymer/lysate solution	Foam generation can occur but should not exceed well top level to avoid cross-contamination.
Magnetic bead carry-over	Minimal carryover might occur. If the solution is to viscous, dilute cell lysate with protein buffer according to absorbance at 280 nm.
Tip does not move centered in the wells	Please contact INTEGRA ASSIST PLUS support for X-axis calibration.
Tip can't aspirate solution at well bottom	Make sure the tips and plates are compatible with the system (see material ID). If that does not solve the problem, please contact INTEGRA ASSIST PLUS support for Z-axis calibration.
Foam or liquid was present above filter in the tip	Please contact INTEGRA ASSIST PLUS support.
Magnetic beads can't be immobilized with the magnet	Cell lysate is too viscous. Dilute cell lysate with protein buffer according to absorbance at 280nm.
When aspirating the final eluate, the end of the tip is filled with air and not liquid	This is normal since the final volume is slightly lower due to multiple volume transfers.
After elution, some magnetic beads are visible at the well wall near the well top.	This is intended. Magnetic beads will be flushed back to the bottom during the second elution.
Solubilization efficiency & yield	
No visible solubilization/ solution remains turbid	Increase temperature stepwise (e.g., 4 °C, RT, 30 °C, 37 °C) and extend the incubation time; higher temperature accelerates membrane insertion and nanodisc formation.
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	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-, FLAG-, or Rho1D4-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, 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 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.