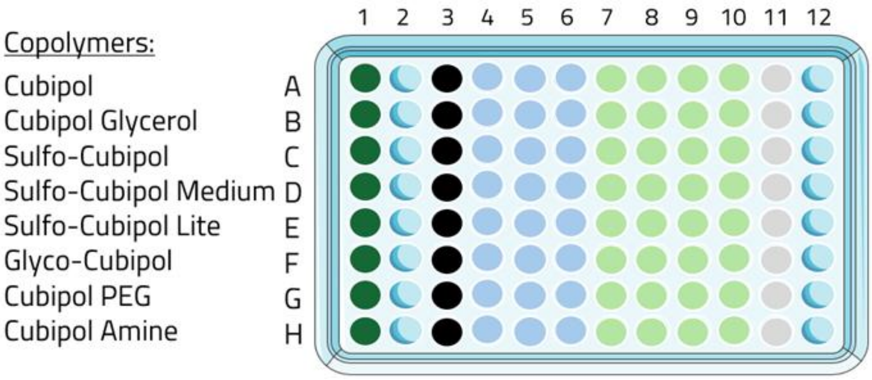
Membrane protein isolation using PlateX MP^{TM} with CyBio FeliX

Automated NativeMP™ copolymer screening and protein purification within one plate

1. Description

Cube Biotech's PlateX MP^{TM} 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$_2$O and cell lysate containing the target protein are additionally required. Three plate variants are available specifically for the Analytik Jena CyBio FeliX: 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^{TM} 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 (Axygen)	90610
PlateX MP™ Anti-DYKDDDDK MagBeads, 96 deep-well plate (Axygen)	90710
PlateX MP™ Strep-Tactin®XT MagBeads, 96 deep-well plate (Axygen)	90810

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

Equipment for CyBio FeliX set up		Cat. No.	Quantity
FeliX CHOICE Workstation	Analytik Jena	OL5015-20-125	1
CyBio TipRack 96/1000 µL PSF	Analytik Jena	OL3812-25-878	1
Support; 37 mm height	Analytik Jena	OL3317-11-120	1
Mounting Kit, BioShake 3000 Series	Analytik Jena	OL3317-23-692	1
TipRack 96/1000 µL	Analytik Jena	OL3317-11-140	1
Gripper incl. 37 mm Support	Analytik Jena	OL3317-14-800	1
CyBio TipBox 96/1000 µL; PSF	Analytik Jena	OL5022-25-579-F	2
CHOICE 8-Channel Adapter, 1-1000 µL	Analytik Jena	OL3316-11-330	1
Adapter 24 tubes, passive cooling function	Analytik Jena	844-00136-0	1
ALPAQUA®, MAGNUM FLX, Universal Magnet Adapter	Analytik Jena	OL3317-11-285	1
QINSTRUMENTS BioShake 3000-T-elm	Analytik Jena	QINSTRUMENTS-2016-0517	1
QINSTRUMENTS Adapter DW – 96 wells v-bottom, 2.2 mL	Analytik Jena	848-2016-1214	1
Reservoir, 100 mL capacity			1
1.5 mL reaction tubes			8

The composition of the recommended protein buffer and the necessary protease inhibitor stock solutions 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$_2$O	-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$_2$O		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 min 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 copolymer 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^{TM} column 2 (Figure 1).

3.2. Set up CyBio FeliX

3.2.1. Fill 100 mL ddH$_2$O in the Reservoir.

3.2.2. Place the Support (Cat. No. OL3317-11-120) on position 6 and remove the cover from the choice head using the “unload” followed by "vertical drive to topmost position".

3.2.3. Remove the cover from the adapter holder.

3.2.4. Please position plates, adapters and tip boxes as follows:

Figure 2: Schematic representation of the CyBio FeliX and the positions (1-12) of the devices within the liquid handler.

#	Device	Cat. No.	Instruction
1	Mounting Kit, BioShake 3000 Series, QINSTRUMENTS BioShake 3000-T-elm and QINSTRUMENTS Adapter DW – 96 wells v-bottom, 2.2 mL	OL3317-23-692 QINSTRUMENTS-2016-0517 848-2016-1214	Ensure cable is free, flexible and unobstructed.
2	TipRack 96/1000 µL	OL3317-11-140	Place tips in wells A1–A8.
3	Gripper incl. 37 mm Support	OL3317-11-800	Position with ID number facing user.
4	CyBio TipBox (96/1000 µL; PSF) and CyBio TipRack (96/1000 µL PSF)	OL5022-25-579-F Tips: OL3812-25-878	Use full tip box (take tips from column 12 for position 2).
5	CyBio TipBox (96/1000 µL; PSF)	OL5022-25-579-F	Use empty tip box to enable tip reusing.
6	CHOICE 8-Channel Adapter, 1-1000 µL	OL3316-11-330	Position with ID number facing user.
7	Empty		
8	Adapter 24 Tubes with eight 1.5 mL reaction tubes	844-00136-0	Place 1.5 mL reaction tubes in A1-A6 and B1-B2.
9	Empty		
10	ALPAQUA®, MAGNUM FLX	OL3317-11-285	Position with ID facing user.
11	PlateX MP$^{\text{TM}}$		Filled with 1.8 mL cell lysate in each well of column 2. Orient plate with A1 in upper-left corner.
12	Reservoir, 100 mL capacity		Filled with 100 mL ddH$_2$O.

3.3. Solubilization, stabilization and purification of membrane proteins

All subsequent steps are performed automatically by CyBio FeliX. Download the complete program and single steps here.

3.3.1. Lyophilized **equilibration and wash buffer** (column 4 to 10) is resuspended with **950 µL ddH$_2$O**.

3.3.2. Lyophilized **elution buffer** (column 11) is resuspended with **250 µL ddH$_2$O**.

3.3.3. **Cubipols** are dissolved with cell lysate. For that, **900 µL cell lysate** are transferred from column 2 in column 1. Cell lysate and copolymer are **mixed for 200 cycles**. Transfer remaining **900 µL cell lysate** are transferred in column 1. Solution is mixed for **10 min**. **Time [sec]** can be set during start message of the script via variable "**Copolymer_mix_time2**".

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 **2 min** for the **following**. **Time [sec]** can be set during start message of the script for the first washing cycle via variable "**Mix_time_beads_2**" and for the following cycles via variable "**Mix_time_beads**". Magnetic beads are separated from buffer by transferring the plate to the magnet. Supernatant is removed and returned to the initial column.

3.3.5. Step 3.3.4. are 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**. **Time [sec]** can be set during start message of the script via variable "**Mix_time_binding**". Magnetic beads are separated from the mixture by transferring the plate to the magnet. Cell lysate-copolymer mixture is returned to column 1 by pipetting twice 900 µL (flow-through fraction).

3.3.7. **Magnetic beads** (column 3) are washed with **950 µL washing buffer**. Washing buffer transfer is started from column 7. Mix Magnetic beads are mixed by pipetting up and down for 2 min. **Time [sec]** can be set during start message of the script via variable "**Mix_time_Washing**". Magnetic beads are separated from the mixture by transferring the plate to the magnet. Supernatant is removed and returned to the initial column.

3.3.8. Step 3.3.7. is repeated **three times**.

3.3.9. Target protein is eluted from magnetic beads (column 3) with **50 µL elution buffer** (column 11). Optional: Volume can be set during start message of the script from 50 µL to 125 µL via variable "**Elution_volume**". Magnetic beads are mixed by pipetting up and down for **5 min**. **Time [sec]** can be set during start message of the script via variable "**Mix_time_elution**". Magnetic beads are separated from the mixture by transferring the plate to the magnet. Eluate is transferred to column 12.

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

3.3.11. **Eluate** is transferred to an **1.5 mL reaction tube** for later use.

4. Troubleshooting

Handling of PlateX MP™ and CyBio FeliX	
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 Analytik Jena CyBio FeliX 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 Analytik Jena CyBio FeliX support for Z-axis calibration.
Foam or liquid was present above filter in the tip	Please contact Analytik Jena CyBio FeliX 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 280 nm.
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.