Membrane protein solubilization & stabilization with copolymers

Stabilization of membrane proteins in native nanodiscs (NativeMP™) for downstream applications

1. Description

Amphiphilic copolymers are used to solubilize and stabilize membrane proteins. In contrast to detergents or membrane scaffolding protein (MSPs) nanodiscs, they enable detergent-free stabilization of membrane proteins in their native lipid environment, contributing to high stability and preserved functionality. However, a challenge for this method is that membrane proteins are very diverse and embedded in individual lipid environments. In addition, predicting the most promising copolymer based on protein sequence or structure is not possible. The choice of copolymer also varies depending on the expression system and on downstream applications. These factors make initial screening for the most effective copolymer key for a successful outcome. The best working copolymer can subsequently be used for scale-up experiments.

This protocol describes how to solubilize & stabilize membrane proteins from different sample types using copolymers.

2. Required material and recommendations

2.1. Copolymers

Different copolymers backbones are available at Cube Biotech, including classical (SMA & DIBMA) and next-generation copolymers (AASTY, Ultrasolute™ Amphipol & Cubipol). All backbones are offered as unmodified version or with different chemical modifications (e.g. glyco-functionalization, glycerol-derived groups, PEGylation or zwitterionic sulfobetaine). For downstream applications that require membrane protein immobilization via streptavidin such as SPR or BLI, we recommend the use of biotinylated copolymers.
To simplify initial screening, Cube Biotech offers screening kits that contain different copolymer variants (see table below).

Cat. No.	Product	Content
18299	NativeMP™ Screening Kit + Innovation Pack	32x different copolymers (broad selection of SMA, DIBMA, AASTY, Ultrasolute™ Amphipol and Cubipol variants)
19791	NativeMP™ Biotin Kit	6x different biotinylated copolymers

2.2. Buffers

Buffer conditions are protein-dependent and should be adapted to the target protein of choice as well as to the affinity tag chosen for subsequent protein purification. A buffer composition as mentioned below can be used for protein purification via Rho1D4-tag, Strep-tag®II/Twin-Strep-tag®, FLAG-tag or His-tag. Please note that we recommend Rho1D4-tag, Strep-tag®II/Twin-Strep-tag® or FLAG-tag for membrane protein purification.

Exemplary protein buffer: 20 mM HEPES pH 7-8.5, 150 mM NaCl

2.3. Further material
Further required material strongly depends on sample property (e.g. cell type used, membrane protein complexity, or sample amount) and planned downstream applications. See 2.4. for recommendations. The table below lists material that might be required, depending on the experimental approach.
Cell lysis buffer (detergent-free)
Protease inhibitors
DNAse/Benzoase
Cell disruptor e.g. sonicator or French press
Ultracentrifuge

2.4. Recommendations
Depending on e.g. experimental aim, cell type, target protein properties or sample amount, it is possible to perform copolymer solubilization & stabilization from different starting materials. You can find our recommendations as well as advantages and disadvantages for all approaches below.

2.4.1. Solubilization from whole cells/crude cell lysate	
Recommended for: initial/small screenings to evaluate e.g. which construct, which copolymer or which buffer conditions are most promising	
Advantages	**Disadvantages**
Short sample preparation time	Cell debris, soluble proteins, DNA (increases viscosity) and irrelevant membranes (e.g. organelles) present in sample → higher total copolymer amount needed compared to pre-purified target membranes
No ultracentrifugation required	Polymer:membrane ratio cannot be determined reliably → contributes to lower reproducibility
Time-saving way to evaluate solubilization and stabilization efficiency	Not suitable for targets with very low expression levels → unspecific background may be high
	Not suitable if highly pure preparation is needed for downstream applications
	Starting from whole cells is not possible for yeast, plants, fungi or gram-positive bacteria

2.4.2. Solubilization from clarified cell lysate	
Recommended for: initial/small screenings to evaluate e.g. which construct, which copolymer or which buffer conditions are most promising.	
Advantages	**Disadvantages**
Short sample preparation time	Irrelevant membranes (e.g. organelles) and DNA present in sample (dependent of lysis technique) → higher total copolymer amount needed compared to pre-purified target membranes
No ultracentrifugation required	Polymer:membrane ratio cannot be determined reliably → contributes to lower reproducibility

Lower amount of contaminants (cell debris) than crude cell lysate	Not suitable for targets with very low expression levels → unspecific background may be high
Time-saving way to evaluate solubilization and stabilization efficiency	Not suitable if highly pure preparation is needed for downstream applications

2.4.3. Solubilization from membrane pellet

Recommended for: large scale (≥ 100 ml cell culture) membrane protein solubilization & stabilization and/or if highly pure protein is needed for e.g. structural or functional downstream analyses.

Advantages	**Disadvantages**
Membrane concentration can be measured and membrane:copolymer ratio determined accurately	Time-consuming sample preparation
Less copolymer used on irrelevant cell fragments/components	Ultracentrifugation is required
Reproducible results	Potential membrane loss during preparation steps
Suitable for very low expressed targets	Multiple sample preparation steps might not be suitable for large/fragile protein complexes or weak protein-lipid interactions
Less unspecific background signals	

3. Protocol

3.1. Copolymer preparation

Initial screening of different copolymers to identify the most effective one for your target membrane protein(s) is recommended.

3.1.1. Prepare a 10% copolymer stock solution by dissolving e.g. 50 mg of copolymer in 500 µl double distilled water.

3.2. Membrane protein solubilization & stabilization starting from whole cells or crude lysate

Initial screening of different copolymers to identify the most effective one for your target membrane protein(s) is recommended.

3.2.1. Collect cells and centrifuge at **400 x g** for **10 min**. Discard supernatant. If you plan to solubilize directly from whole cells, continue with 3.2.4.

3.2.2. Add at least **3 ml** buffer (see 2.2. for recommendation) containing a protease inhibitor cocktail (e.g. 0.01 mM leupeptin, 0.01 mM E-64, 0.1 mM PMSF, 1 mM pepstatin, 1 mM phenanthroline) **per ml** cell pellet.

3.2.3. Disrupt the cells with e.g. sonication (e.g. **3 cycles, 5 min** each) on ice.

Optimal sonication time and power depend on sample volume and type of sonicator. Make sure that sample temperature remains low. Other cell lysis methods such as French press can also be used.

3.2.4. *Optional:* Take a small sample (around **5 µl**) of the cell lysate and dilute it **1/100** in protein buffer. For Blank, dilute protein buffer containing protein inhibitors in the same way.

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

3.2.5. Add copolymer stock solution to the resuspended cell pellet (resuspension buffer should include protease inhibitors and DNAse/Benzoase) or crude lysate at a final concentration of **2.5%** (e.g. add 250 µl of the 10% stock solution prepared in 3.1.1. to 750 µl cell suspension or 750 µl crude lysate). Mix thoroughly.

A concentration of 2.5% can be used as a starting point for initial screening experiments and to evaluate copolymer performance. Further titration of optimal concentrations might be necessary.

3.2.6. Solubilize by stirring at temperature of choice (**from 4 °C to 37 °C**) until solution clears visibly. This can range from **15 min to several hours** depending on membrane- and cell type. A higher temperature correlates with a shorter solubilization time.

3.2.7. *Optional:* Centrifuge sample at **15,000 x g** for **2 min** to separate cell fragments. Collect the supernatant in a new tube.

3.2.8. The sample is now ready for purification via the chosen affinity tag and subsequent further downstream applications.

3.3. Membrane protein solubilization & stabilization starting from clarified cell lysate

Initial screening of different copolymers to identify the most effective one for your target membrane protein(s) is recommended.

3.3.1. Collect cells and centrifuge at **400 x g** for **10 min**. Discard supernatant.

3.3.2. Add at least **3 ml** buffer (see 2.2. for recommendation) containing a protease inhibitor cocktail (e.g. 0.01 mM leupeptin, 0.01 mM E-64, 0.1 mM PMSF, 1 mM pepstatin, 1 mM phenanthroline) **per ml** cell pellet.

3.3.3. Disrupt the cells with e.g. sonication (e.g. **3 cycles, 5 min** each) on ice.

Optimal sonication time and power depend on sample volume and type of sonicator. Make sure that sample temperature remains low. Other cell lysis methods such as French press can also be used.

3.3.4. Centrifuge the cell lysate at **9,000 x g** for **45 min**. Transfer the supernatant (clarified cell lysate) to a new tube.

3.3.5. *Optional:* Take a small sample (around **5 µl**) of the supernatant and dilute the sample **1/100** in protein buffer. For Blank, dilute protein buffer containing protein inhibitors in the same way.

Measure the absorbance of the diluted sample at **280 nm** and calculate the absorbance of the undiluted cell lysate. 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.

3.3.6. Add copolymer stock solution to the clarified cell lysate at a final concentration of **2.5%** (e.g. add 250 µl of the 10% stock solution prepared in 3.1.1. to 750 µl cell lysate). Mix thoroughly.

A concentration of 2.5% can be used as a starting point for initial screening experiments and to evaluate copolymer performance. Further titration of optimal concentrations might be necessary.

3.3.7. Solubilize by stirring at temperature of choice (**from 4°C to 37°C**) until solution clears visibly. This can range from **15 min to several hours** depending on membrane- and cell type. A higher temperature correlates with a shorter solubilization time.

3.3.8. The sample is now ready for purification via the chosen affinity tag and subsequent further downstream applications.

3.4. Membrane protein solubilization & stabilization starting from membrane pellet

Initial screening of different copolymers to identify the most effective one for your target membrane protein(s) is recommended.

3.4.1. Collect cells and centrifuge at **400 x g** for **10 min**. Discard supernatant.

3.4.2. Add at least 3 ml buffer (see 2.2. for recommendation) containing a protease inhibitor cocktail (e.g. 0.01 mM leupeptin, 0.01 mM E-64, 0.1 mM PMSF, 1 mM pepstatin, 1 mM phenanthroline) per ml cell pellet.

3.4.3. Disrupt the cells with e.g. sonication (e.g. **3 cycles, 5 min** each) on ice.

Optimal sonication time and power depend on sample volume and type of sonicator. Make sure that sample temperature remains low. Other cell lysis methods such as French press can also be used.

3.4.4. Centrifuge the cell lysate at **9,000 x g** for **30 min**.

3.4.5. Weigh the tube intended for collection of membranes via ultracentrifugation. Transfer the supernatant (clarified cell lysate) to this tube.

3.4.6. Centrifuge clarified cell lysate at **100,000 x g** for **45 min** to collect membranes.

3.4.7. Weigh the tube to determine the amount of collected membranes.

3.4.8. Dilute copolymer stock solution to a final concentration of **2.5%** in a suitable buffer for your target protein of choice (see 2.2. for recommendations).

3.4.9. Add **15 ml** of the prepared copolymer solution **per gram** of collected membranes.

A concentration of 2.5% can be used as a starting point for initial screening experiments and to evaluate copolymer performance. Further titration of optimal concentrations might be necessary.

3.4.10. Sonicate the suspension **3x** for **3 min** to enhance solubilization. Incubate while stirring for **2 h** at RT.

Optimal sonication time depends on sample volume and type of sonicator. Make sure that sample temperature does not get too warm. Other cell lysis methods such as French press can also be used.

3.4.11. Centrifuge sample at **100,000 x g** for **45 min** to remove insoluble membranes. Transfer supernatant containing solubilized membranes to a new tube.

Weigh the tube containing insoluble material to determine the solubilization efficiency.

3.4.12. The sample is now ready for purification via the chosen affinity tag and subsequent further downstream applications.

4. Troubleshooting

4.1. Solubilization efficiency & yield	
Problem	**Solution**
No visible solubilization / solution remains turbid	Increase temperature stepwise (4 °C → RT → 30 °C → 37 °C) and extend incubation; higher temperature accelerates membrane insertion and nanodisc formation.
Very low membrane protein yield	Perform a NativeMP™ screening across multiple copolymer backbones; copolymer compatibility is target- and lipid-environment dependent and cannot be predicted.
Protein present in pellet after ultracentrifugation	Perform a NativeMP™ screening across multiple copolymer backbones; copolymer compatibility is target- and lipid-environment dependent and cannot be predicted.
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 mis-localized proteins.
4.2. Protein stability, function & homogeneity	
Problem	**Solution**
Protein solubilized but unstable over time	Switch copolymer backbone (e.g. SMA → DIBMA, AASTY, Ultrasolute, Cubipol); instability often reflects polymer–lipid mismatch rather than intrinsic protein instability.
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.
Protein degrades during solubilization	Strengthen protease inhibitor cocktail, reduce processing temperature, and shorten handling time; copolymers do not inactivate proteases.
4.3. Background, purity & sample quality	
Problem	**Solution**
Sample becomes highly viscous	Add DNAse/Benzoase before copolymer addition; copolymers do not remove nucleic acids and viscosity reduces effective solubilization.
4.3. Copolymer handling & chemistry	
Problem	**Solution**

Copolymer precipitates during preparation	Ensure complete dissolution of 10% stock and adjust pH to neutral range after protease addition; partial protonation strongly affects solubility. Check divalent ion concentration and polymer compatibility if present in buffer.
Copolymer solution has a yellow appearance	This is normal and does not effect product efficiency
4.4. Reproducibility, scale-up & workflow choice	
Problem	**Solution**
Poor reproducibility between experiments	Avoid whole-cell solubilization for optimization; membrane pellets allow accurate polymer:membrane ratios. Control expression, buffers, incubation times, and elution conditions strictly.
High target protein loss during membrane pellet preparation	Reduce mechanical stress and avoid excessive centrifugation; fragile lipid–protein assemblies can be lost before solubilization. Whole-cell or lysate workflows may be preferable.
Different copolymers work in screening but fail at scale	Re-optimize polymer:membrane, 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.