Kit for the fast and easy Extraction of Membrane Proteins from Eukaryotic Cells and Tissues

Kit contents (sufficient for 130 extractions of up to 300 µg protein)

- 800-MXK A: SySy MemEx Reagent A, 10 ml
- 800-MXK B: SySy MemEx Reagent B, 30 ml
- 800-MXK C: SySy MemEx Reagent C, 10 ml
- 800-MXK D: SySy MemEx Dye, 300 µl

Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature which may cause Reagent A to appear cloudy upon receipt. Storage at 4°C will clarify the solution. Keep Reagent A at 4°C or on ice at all times during usage of the kit. Reagent C precipitates at 4°C and should be dissolved by warming before use.
Shelf life: 6 months

Materials and reagents

- **Water bath**
- **Ice**
- **Protease inhibitors:** e.g. 1 mM PMSF / 1 µg/ml Aprotinin / 2.5 µM Pepstatin A / 2 mM EDTA or commercialy available cocktails.

General information

SySy MemEx employs a detergent-based temperature-dependent phase separation to enrich membrane proteins from mammalian tissue or cell homogenates. While most membrane proteins partition to the detergent phase with 80-90% efficiency, the soluble proteins generally remain in the aqueous phase. Although the extraction protocol includes a centrifugation step to remove insoluble proteins and nuclei, only ultracentrifuation can pellet all insoluble content.

Important product information

For optimal results, add **protease inhibitors** to your cell suspension or tissue homogenate. If you have not regularly performed phase separations yet, you should add MemEx dye as indicated which will stain the membrane protein phase and will help you to discriminate between the phases. Perform 37°C incubation step in a water bath since incubator ovens or heating blocks do not heat evenly enough for this application. Do not process too many parallel reactions in one extraction procedure because resolubilization of the hydrophobic phase into the hydrophilic phase occurs at room temperature. Both the aqueous phase and the resuspended detergent phase can be extracted a second time to increase purity and yield (aqueous phase: start over with step 2, detergent phase: start over with step 3).

Starting material

The procedure can be applied to mammalian cells or tissues or to any other homogenized protein suspension. A concentration of 0.5% Triton X-100 in the buffer, e.g. after mild cell lysis, is tolerated but may decrease the yield of the extraction.

Procedure

1. Adjust the starting material to 1-4 mg/ml protein with Reagent B (suggested sample volume 75 µl).
2. Add an equal volume of Reagent A and (optional) 1/50 volume of the dye solution.
3. Incubate at 4°C for 30 min to 1 hour.
4. Pellet the insoluble fraction at 4°C and maximum speed for 30 min in a tabletop centrifuge (should be replaced by ultracentrifugation at 100,000x g for 30 min, if available).
5. Transfer the tubes to 37°C and incubate for 10 min.
6. Spin in a tabletop centrifuge at 10,000 rpm (approx. 9,000x g) at room temperature for 3 min.
7. Carefully remove the upper aequous phase and eventually transfer it to a new tube for analysis.
8. The lower detergent phase contains enriched membrane proteins and should be resuspended with 1.6 volumes of cold Reagent B (e.g. 120 µl) or another suitable buffer for downstream applications.

Troubleshooting

My protein of interest is neither predominantly found in the aqueous phase nor in the detergent phase

- Test all fractions including starting material and insoluble fraction.
- If the protein is mainly insoluble, addition of 1/10 volume Reagent C and DNase at 0.1 µg/µl to the starting material followed by a 10 min incubation at room temperature before addition of Reagent A (step 2) may help to increase the yield in the detergent phase. Leave out EDTA as protease inhibitor. Add Reagent C as the last component. Note that this variation will lead to some contamination of the detergent phase with soluble proteins.
- If you cannot find your protein of interest except in the starting material, you should increase the concentration of protease inhibitors.

The protein of interest is found mainly in the detergent phase, but some of it remains in the aqueous phase

- The aqueous phase (step 7) can be extracted a second time to collect the remaining protein (start again with step 2). However, a few membrane proteins do not completely segregate with the detergent phase.

Supporting protocols

Homogenization of cells or tissues

1. Resuspend cells or tissues in 2 µl or 10 µl Reagent B per mg material, respectively, including protease inhibitors.
2. Disrupt and homogenize cells, e.g. by passing through a needle or by sonication. Disrupt and homogenize tissues, e.g. by a rotor-stator mixer.
3. Determine the protein concentration by a protein estimation like Bradford assay. Dilute at least twofold for extraction.

Methanol-chloroform precipitation for detergent removal and sample concentration, e.g. to perform Western Blotting

1. Add 4 volumes of methanol to the sample and vortex vigorously.
2. Add 2 volumes of chloroform and vortex vigorously.
3. Add 3 volumes of water and vortex vigorously.
4. Spin at maximum speed in a table-top centrifuge for 5 min.
5. Carefully remove the upper face without disturbing the interface (contains white protein precipitate)!
6. Add 3 volumes of methanol, vortex vigorously and spin again.
7. Discard the supernatant and dry the precipitate under a hood for 5 min (or until no liquid remains. Too long incubation will make the pellet difficult to resuspend!).
8. Resuspend in 0.1-1 volume of SDS sample buffer or another suitable buffer (if necessary, agitate at 37°C).

Acetone precipitation for detergent removal and sample concentration, e.g. to perform Western Blotting

1. Add 4 volumes of -20°C acetone to the sample and vortex vigorously.
2. Incubate for 30 min to 4 h at -20°C.
3. Spin at maximum speed in a table-top centrifuge for 10 min.
4. Discard the supernatant and dry the precipitate under a hood for 5 min (or until no liquid remains. Too long incubation will make the pellet difficult to resuspend!).
5. Resuspend in 0.1-1 volume of SDS sample buffer or another suitable buffer (if necessary, agitate at 37°C).