

# Preparation of detergent-solubilized membranes from *Escherichia coli*

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## Method Article

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## Abstract

This protocol describes a method to prepare detergent-solubilized membranes from *Escherichia coli* (E. coli), e.g. containing an overexpressed membrane protein. The procedure takes less than one day. Cells are broken by pressure cell and membranes are isolated and washed by differential centrifugation. Finally, the membranes are solubilized with the detergent of choice.

## Reagents

Cell pellet of *E. coli*, Tris, EDTA, NaCl, n-dodecyl- $\beta$ -D-maltoside (DDM) or another detergent of choice, glycerol, NaN<sub>3</sub>

## Equipment

French Press, ultracentrifuge

## Procedure

1. Resuspend the cell pellet from 1 l of *E. coli* culture in Lysis Buffer (20 mM Tris-HCl, pH 8.0, 0.5 mM EDTA). Adapt the buffer volume to your French pressure cell.
2. Disrupt *E. coli* cells by passage through a French pressure cell (20,000 psi) and remove unbroken cells by centrifugation at 10,000g (4°C, 10 min).
3. Ultracentrifuge the supernatant at 100,000g (4°C, 1 h).
4. Resuspend and homogenize the pellet containing the *E. coli* membranes in Lysis Buffer and ultracentrifuge again.
5. Remove water-soluble proteins adhering to the membrane by homogenization in 20 mM Tris-HCl, pH 8.0, 300 mM NaCl and ultracentrifugation. Breakpoint: Membrane pellet can be resuspended in 1 ml buffer without detergent and stored at -80 °C for months to years prior detergent solubilization.
6. Resuspend and solubilize the membrane pellet in 1% DDM, 20 mM Tris-HCl, pH 8.0, 300 mM NaCl, 10% glycerol, 0.01% NaN<sub>3</sub> for 2 h at 4°C under gentle agitation (final volume: 7 ml).
7. Ultracentrifuge at 100,000g (4°C, 50 min).
8. The supernatant represents solubilized membranes, which can be used for purification of the His-tagged protein or SPA-binding experiments directly.

## Timing

The procedure takes less than one day.

## References

Casagrande, F. et al. Projection structure of a member of the amino acid/polyamine/organocation transporter superfamily. *J. Biol. Chem.* 283, 33240-33248 (2008).