

May 03, 2024

Database-driven protein solubilization

DOI

<https://dx.doi.org/10.17504/protocols.io.36wgqn735gk5/v1>

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Protocol Citation: Caroline Brown, Snehasish Ghosh, Kallol Gupta 2024. Database-driven protein solubilization. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.36wgqn735gk5/v1>

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Protocol status: Working

We use this protocol and it's working

Created: May 03, 2024

Last Modified: May 03, 2024

Protocol Integer ID: 99218

Keywords: membrane proteins into native nanodisc, driven protein solubilization, protein solubilization, membrane protein, extraction database, protein, membrane, extraction, optimal extraction, native nanodisc

Abstract

This is a protocol for using the extraction database found at www.polymerscreen.yale.edu for optimal extraction of membrane proteins into native nanodiscs.



- 1 Choose your protein of interest.
- 2 Visit www.polymerscreen.yale.edu to search for your protein in the extraction database. This will provide you with the polymer to use for optimal protein extraction.
- 3 As an example, below are representative organellar markers and the optimal polymer for extraction:
TMEM192 → AASTY650
TGN46 → ChloroSMA60
OMP25 → SMA200
VAPA → AASTY650
KRas → ChloroSMA80
- 4 Once the optimal polymer has been determined, express the protein of interest, or harvest cells for endogenous solubilization.
- 5 Resuspend cellular membranes in the chosen polymer and incubate at  4 °C with rotation for  02:00:00 hours. 2h
- 6 Ultracentrifuge samples at 200,000xg for  01:00:00 hours to remove insoluble material. 1h