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Membrane Fractionation

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1 Works for me

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ABSTRACT

Membrane Fractionation (Ultracentrifugation)

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PROTOCOL CITATION

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- 1 seed cells for 24:00:00 in a 10 cm dish at a density of 6×10^6 cells per plate 1d
- 2 wash cells with 1X PBS for 2 times
- 3 scrape cells of the dish and harvest in 1 ml pf PBS + PI buffer
- 4 1000 x g, 00:15:00 15m
- 5 resuspend cells in 500 μ l PBS + PI buffer

- 6 sonicate 2 times at 30Hz for 15 ON-OFF intervals of 10 s each (1 s pulse on/off).
- 7  **340000 x g, 4°C, 00:15:00** 15m
collect supernatant as cytosolic fraction
- 8 was pellet 3 times with PBS + PI buffer
- 9  **340000 x g, 4°C, 00:15:00** 15m
- 10 dissolve pellet in  **500 µl** 1x PBS +PI+ 1% Trtiton
- 11  **340000 rpm, 4°C, 00:15:00** 15m
collect supernatant as membranous fraction