Membrane Fractionation

Veerle Baekelandt[1]
[1]KU Leuven

Aug 05, 2021

dx.doi.org/10.17504/protocols.io.bw5vpg66

Joris Van Asselberghs

ABSTRACT

Membrane Fractionation (Ultracentrifugation)

DOI

dx.doi.org/10.17504/protocols.io.bw5vpg66

PROTOCOL CITATION

Veerle Baekelandt 2021. Membrane Fractionation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bw5vpg66

CREATED

Aug 05, 2021

LAST MODIFIED

Aug 05, 2021

PROTOCOL INTEGER ID

52117

1 seed cells for **24:00:00** in a 10 cm dish at a density of 6 x 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 µl 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 disolve pellet in **500 µl** 1x PBS +PI+ 1% Trtiton

11 **340000 rpm, 4°C, 00:15:00** 15m
collect supernatant as membranous fraction

Citation: Veerle Baekelandt (08/05/2021). Membrane Fractionation. https://dx.doi.org/10.17504/protocols.io.bw5vpg66