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Protocol status: Working
We use this protocol and it's working

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Microsomal membrane isolation from cell culture

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ABSTRACT

Microsomal membrane isolation from cell culture

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82702

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harvest cells

1 harvest cells by detachment with TrypLE Express (Gibco, 12604-021)

2 pellet cells by centrifugation (5 min, 400 g_{avg}, 4°C)

3 wash cell pellet in phosphate buffered saline (PBS, Sigma, D8537-500ml)

4 pellet cells by centrifugation (5 min, 400 g_{avg}, 4°C)

lyse cells

5 resuspend pellet in RIPA lysis buffer (Thermo Scientific, 89900) supplemented with protease inhibitor (Sigmafast, Sigma, S8830-20TAB)

6 incubate 30min on ice

7 centrifuge lysate (10min, 100 g_{avg}, 4°C) to pellet and remove nuclear fraction

8 collect supernatant to continue isolation

isolate microsomal fraction

9 centrifuge supernatant from previous step (10min, 15 000 g_{avg}, 4°C) to pellet the mitochondrial/lysosomal fraction

10 collect supernatant to continue isolation (pelleted mitochondrial/lysosomal fraction can be stored for future analysis)

11 centrifuge supernatant from previous step (35 min, 200 000 g_{avg}, 4°C) to pellet the microsomal fraction

12 resuspend pellet in sucrose (0.25 M)

13 for Western blotting prepare samples with 20µg protein with 4x LDS loading buffer (NuPAGE, invitrogen, 2399420)