

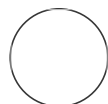


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## 🌐 Membrane and cytosol fractionation

wusj<sup>1</sup>, schekman<sup>1</sup>

<sup>1</sup>Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, United States



Nancy C. Hernandez Villegas

### ABSTRACT

This protocol describes membrane and cytosol fractionation of cells expressing different DNAJC5 isoforms

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




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## Cytosol fractionation



- 1 Cells (one 10 cm dish) were cultured to 70% confluence and transfected with different constructs of DNAJC5
- 2 One day after transfection, we harvested the transfected cells by scraping in 1 ml B88 (20 mM HEPES-KOH, pH 7.2, 250 mM sorbitol, 150 mM potassium acetate, and 5 mM magnesium acetate) plus a cocktail of protease inhibitors
- 3 Cells were homogenized by 10 passages through a 22G needle
- 4 Homogenates were centrifuged at 500×g for  00:10:00 and the resulting post-nuclear supernatant (PNS) fractions were centrifuged at 100,000×g for  01:30:00 1h 40m
- 5 High-speed supernatant fractions were then subjected to a repeat centrifugation to achieve a clarified cytosol fraction
- 6 The pellet fraction was washed and resuspended in the same volume of B88
- 7 Resuspended material was also centrifuged again to collect a washed membrane fraction
- 8 Membranes were lysed in lysis buffer

## Membrane fractionation


- 9 The PNS was subjected to differential centrifugation at 3000×g for  00:10:00 10m
- 10 The supernatant was centrifuged at 25,000×g for  00:20:00 20m
- 11 The supernatant was centrifuged at 100,000×g for  00:30:00 30m
- 12 Membrane fractions were normalized to phosphatidylcholine content and analyzed by immunoblot

## Proteinase K protection assays


25m

- 13 The 25,000×g membrane fraction was aliquoted into three tubes: one without proteinase K, one with proteinase K (10 µg/ml), and one with proteinase K plus TritonX-100 (0.5%)
- 14 The incubation was conducted  On ice for  00:20:00 20m
- 15 The reaction was stopped by sequential addition of PMSF (1 mM)

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Add sample buffer. Then, samples were then heated on metal block at  95 °C for

5m

 00:05:00 and analyzed by SDS-PAGE and immunoblot.