May 03, 2024

Step-centrifugation Assay

DOI
dx.doi.org/10.17504/protocols.io.36wgqn75xgk5/v1

Caroline Brown[1,2,3], Snehasish Ghosh[1,2,3], Kallol Gupta[1,2,3]

[1]Nanobiology Institute, Yale University, West Haven, CT, USA;
[2]Department of Cell Biology, Yale University School of Medicine, New Haven, CT, USA;
[3]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

C Caroline Brown
Yale University

DOI: dx.doi.org/10.17504/protocols.io.36wgqn75xgk5/v1

Protocol Citation: Caroline Brown, Snehasish Ghosh, Kallol Gupta 2024. Step-centrifugation Assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqn75xgk5/v1

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

Created: May 02, 2024

Last Modified: May 03, 2024

Protocol Integer ID: 99142

Abstract

This protocol details the protocol for conducting a step-centrifugation assay to determine the minimum required speed for full separation of vesicles from native nanodiscs.

1 Resuspend cells in lysis buffer ([M] 50 millimolar (mM) Tris HCl pH 7.4 , [M] 150 millimolar (mM) NaCl , [M] 10 % volume glycerol) and lyse using nitrogen cavitation (750 PSI for 00:15:00 minutes). 15m

2 Centrifuge lysed cells at 4000rpm for 00:10:00 minutes to pellet cell debris. 10m

3 Spin the supernatant in a series of sequential ultracentrifugation steps at speeds of 0 µL 20,000xg, 100,000xg, 150,000xg, and 200,000xg with 01:00:00 hour for each spin. 1h

4 After each spin subject the sample to dynamic light scattering to calculate population size distribution.