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Step-centrifugation Assay

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We use this protocol and it's working

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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 μ L 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.