CENTROSOME ISOLATION

PROTOCOL:

1. Spin 5 x 10^9 cells at 500 g for 5 minutes at 4°C.
2. Wash 3X phosphate buffer (5 fold volume of the pellet).
3. Spin for 5 minutes at 500 g at 4°C. Add 2 μM of cytochalasin A or D prior to last step.
4. Resuspend cells in 20 mL of lysis buffer and vortex for 1 minute.
5. Filter suspension through 5 μm nucleopore filter and immediately centrifuge for 10 minutes at 2500 g at 4°C.
6. Discard supernatant and resuspend in 20 mL P Phosphate buffer.
7. Vortex for 1 minute and spin for 10 minutes at 2500 g at 4°C.
8. Discard pellet and add the heparin (6 mg/600 μL H_2O). Set on ice for 5 minutes.
9. Fill 2 SW-40 tubes with 0.5 mL of 8O% SUC and later 1 mL of 50% SUC.
10. Filtrate supernatant through 5 μm nucleopore-filter and layer 1/2 on each tube.
11. Spin for 1 hour at 40,000 g (21000 rpm) at 4°C.
12. Prepare the 2nd gradient in SWSO Beckman tubes: 0.5 mL, 80% SUC
1 mL 55% of SUC and 1 mL of 50% of SUC
13. Remove the upper half of the 50% fraction and resuspend the rest. Pass the suspension 2X by a 27 gauge needle and supplement with half a fold of gradient buffer.
14. Load suspension on the 2nd gradient.
15. Spin at 40,000 g for 1 hour at 4°C.
16. Collect fractions from the bottom using a peristaltic pump and a glass capillary (~200 μL/mm) Fraction should be about 700 μL.