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We use this protocol and it's working

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Medium fractionation and EV preparation

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ABSTRACT

This protocol describes the characterization of the extracellular alpha-synuclein and DNAJC5

MATERIALS

For specific information about reagents and materials to perform an EV preparation, refer to a previous paper published by our lab www.bio-protocol.org/e3706

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Medium fractionation

1 Centrifuge the conditioned medium at 1,500 x g for 00:20:00 at 4 °C in a Sorvall RC 6+ centrifuge with fixed angle rotor F14S-6X250y FiberLite 20m

2 Pour the supernatant into a new container without disturbing the pellet.

3 Centrifuge the supernatant at 10,000 x g for 00:30:00 at 4 °C in a Sorvall RC 6+ centrifuge with fixed angle rotor F14S-6X250y FiberLite 30m

4 The supernatant fractions at each step were collected and treated with methanol/chloroform to precipitate proteins which were then collected by centrifugation

5 Pellet fractions were resuspended in sample buffer to achieve a 20-fold concentration.

6 The sedimented fractions at each step were also collected and resuspended in sample buffer.

7 All the fractions were analyzed by immunoblot.

Extracellular vesicle (EV) preparation

8 Centrifuge the conditioned medium at 1,500 x g for 00:20:00 at 4 °C in a Sorvall RC 6+ centrifuge with fixed angle rotor F14S-6X250y FiberLite 20m

9 Pour the supernatant into a new container without disturbing the pellet.

10 Centrifuge the supernatant at 10,000 x g for 00:30:00 at 4 °C in a Sorvall RC 6+ centrifuge with fixed angle rotor F14S-6X250y FiberLite 30m

11 Pour the supernatant into a new container without disturbing the pellet.

12 Add 35 ml of the collected supernatant into a single 38.5 ml ultra-clear tube. Repeat this step 5 times to fill a total of six 38.5 ml ultra-clear tubes.

13 Centrifuge at ~100,000 x g (29,500 RPM) for 01:30:00 using a SW32 Ti rotor at 4 °C a 1h 30m maximum acceleration and brake.

14 Aspirate the supernatant, put the tubes upside down on paper towel to dry any residue of medium. Aspirate the residue by vacuum system if needed.

15 Resuspend the pellet by adding 500 uL of PBS (pH 7.4) buffer to the bottom of each tube, put the tubes on a compatible rack, and rigorously shake the tubes on orbital shaker in cold room for 30m

00:30:00 .

16 Combine the resuspend pellet (~500 uL x 6 tubes = 3mL; +1mL PBS) and centrifuge again at 120,000 x g (36,500 RPM) x 01:00:00 at 4 °C in SW-55 rotor 1h

17 Resuspend the pellet again in 200uL PBS Buffer and add 1mL of 60% (1.8M) sucrose buffer (20mM Tris 7.4, 150 mM NaCl) and vortex to mix the sample evenly. The final sucrose concentration should be above 50% measured by refractometer.

18 Aliquots of 40% (5 ml) and 10% (2 ml) sucrose buffer were sequentially overlaid above the sample. The tubes were then centrifuged at 150,000×g for 16:00:00 at 4 °C in an SW41 Ti swinging-bucket rotor (Beckman Coulter) 16h

19 After centrifugation, 0.5 ml fractions were collected from top to bottom and samples were analyzed by SDS-PAGE and immunoblot.