Isolation of small extracellular vesicles from cell culture supernatant

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Method Article

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Abstract

This protocol describes how to isolate small extracellular vesicles (sEV) from cell culture media using an ultracentrifuge and analyze the number of sEV by nanoparticle tracking analyzer.

Introduction

Small extracellular vesicles (sEV) are heterogeneous small vesicles secreted by all kind of cells. The sEV, emerging players of systemic factors, play an important role in establishing premetastatic niche in future metastatic organs. Therefore, inhibition of sEV secretion from cancer cells could become very important in reducing the cancer progression and metastasis. To evaluate the efficacy of a sEV secretion inhibitor, sEV were isolated using an ultracentrifuge and analyzed by nanoparticle tracking analyzer (NTA). We report a protocol for reproducibility between users. This protocol describes the detailed method, including the concentration of DMSO in SFX stock, the starting cell number, the size of plates, the volume of culture supernatant, the time of centrifugation and the condition of NTA measurement. All relevant data of our experiments are detailed in a manuscript submitted for publication (Im, E., *et al.* Sulfisoxazole inhibits the secretion of small extracellular vesicles by targeting the endothelin receptor A. *Nat Commun* 10, 1387 (2019)).

Reagents

DMEM/High glucose (HyClone, SH30243.01)

Antibiotic/Antimycotic Solution 100x (HyClone, SV30079.01)

FBS (HyClone, SH30084.03)

PBS (HyClone, SH30256.01)

Trypsin-EDTA (HyClone, SH30042.02)

Sulfisoxazole (Sigma, 31739 and S6377)

GW4869 (Sigma, D1692)

DMSO (PanReac AppliChem, A3672)

Equipment

Centrifuge (Hanil Scientific, Combi R515)

NanoParticle Tracking Analyzer (NanoSight, LM10, software version 2.3)

Ultracentrifuge (Beckman Coulter, Optima XE-90)

Ultracentrifuge rotor (Beckman Coulter, SW28)

Ultracentrifuge tube (Beckman Coulter, 344058)

150-mm plate (SARSTEDT, 83.3903)

50mL tube (Greiner bio-one, 227261)

0.22 µm syringe Filter (Sartorius Stedim Biotech, 16534)

1mL syringe (BD Bioscience, 301321)

10mL syringe (BD Bioscience, 302932)

CO2 incubator (Thermo Forma, 371)

Procedure

Step 1: Seed the 5×10^6 MDA-MB-231 cells per 150-mm plate in 20mL DMEM with 10% FBS, 1% AA (2 plate per group).

Step 2: After 24h, wash using PBS and then change the media (FBS free, AA free) with vehicle or 100µM SFX (Final DMSO concentration: 0.1%).

Step 3: After 24h, collect the cell culture supernatant (40mL).

Step 4: Centrifuge at 300 x g, 4°C, 3min and transfer the supernatants to new tube (39mL).

Step 5: Centrifuge at 2,500 x g, 4°C, 20min and transfer the supernatants to new tube (38mL).

Step 6: Centrifuge at 10,000 x g, 4°C, 30min and transfer the supernatants to new tube (37mL).

Step 7: Filter the supernatants using 0.22 µm syringe filters and transfer the supernatants to Ultra-Clear tube (#344058, Beckman Open-Top Tube).

Step 8: Ultracentrifuge at 120,000 x g, 4°C, 90min.

Step 9: Resuspend sEV pellets with 30mL PBS and centrifuged at 120,000 x g, 4°C, 90min again.

Step 10: Remove the supernatants and add the 1ml PBS.

Step 11: After seal tube with parafilm, store at 4°C, 1h and resuspend the pellet.

Step 12: Transfer the samples to e-tube and analyze using nanosight (Samples were analyzed at camera level of 9 and detection threshold of 4.).

Troubleshooting

An optimal concentration of 50-200 particles/frame allows for sufficient data collection.

Time Taken

Anticipated Results

References

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