Experimental Protocol

Exosomes isolation protocol from cell cultures media by ultracentrifugation

Total time for experiment: 3h 30min

Reagents:

PBS 1x (pre-filtered)
Depleted-FBS (vesicles free)
2-hydroxyethyl methacrylate
RPMI cell culture medium supplemented with AAs and P/S
BSA and growth factors

Instruments and labware:

Refrigerated Centrifuge, capable of ~3000g with a swing bucket rotor
Pipetting aid or plastic disposable bulb pipettes
P100, P200 and P10 pipettes
P100, P200 and P10 filter tips
0.1 and 0.2 µm filters
20 and 50ml Luer-Lock syringes
1ml Eppendorf tubes
10ml and 25ml serological disposable pipettes
50ml polypropylene centrifuge tubes
Cell Culture Flask T175
-20 and -80^{o}C Temperature Freezer
Ultracentrifuge and aproppiate tubs and caps
Freezer storage boxes for 1-2ml cryogenic vials

Prior considerations:

The amount of cells needed to carry out a protocol for obtaining exosomes is determined based on the growth rate of each culture; the cell confluence cannot exceed 80% of the surface of the flask (175 cm2) at 72 hours after seeding.
The culture medium used for cell growth depending on the type of culture. For growth under adhesion conditions, it is necessary in the medium content FBS (fetal bovine serum) previously

depleted of microvesicles by ultracentrifugation and filtering. The culture of the cells in suspension is carried out in bottles of low adhesion, covered with poly (2-hydroxyethyl methacrylate), used without FBS and supplemented with BSA and growth factors that favor the proliferation of cells with cancer stem cells properties. RPMI medium supplemented with AA and P / S is used for both types of culture. The culture conditions are at 37 ° C and 5% CO2. The cells are seeded with a total volume of 30 ml of culture medium.
Once the 72 hours have elapsed after sowing, a centrifugation protocol is carried out with the objective of eliminating cell debris, apoptotic bodies and other macrovesicles that may interfere in the isolation of exosomes.

Steps:

Centrifugation and cleaning protocol:

1) Centrifuge at 500 g for 5 minutes at 4 ° C.

2) Transfer in the culture medium to another conical tube and centrifuge again at 3,000 g for 15 minutes at 4°C.

3) Filter the supernatant with a 0.22 µm filter.

4) Centrifuge at 17,000 g for 10 minutes at 4 ° C.

Subsequently, the culture medium can be frozen at -80°C for less than 7 days or at 4°C for the isolation in the following 24h.

The ultracentrifugation procedure for exosomes isolation is performed at 110,000 g for 1:45h at 4°C. Once exosomes pellet is obtained, it is resuspended in 60 µl of pre-filtered PBS and frozen at -20°C for further applications. In the case that the samples are used in imaging techniques, it is necessary to purify them again in 30 ml of PBS and re-centrifuge at 110,000 g for 1:45h at 4°C.

Contact:

liquidbiopsy@ciberonc.es