SOP: SP069

Production of CFP-Containing Exosomes

Materials and Reagents:

1. Activated culture of THP1 monocyte cells (see SOP: SP058 & SOP: SP068)
2. 37 °C incubator with 5% CO_2
3. Water bath at 37 °C
4. RPMI 1640-Complete (see SOP: M023)
5. CFP (Culture Filtrate Protein)
6. Table top centrifuge
7. Inverted Microscope
8. Tissue culture/biosafety cabinet
9. Serological pipettes
10. Falcon tubes 50 mL
11. Pipet tips/pipetman
12. 0.22 μm syringe filter
13. Sterile syringe
14. Pipette-Aid

Protocol:

1. ____Prepare the tissue culture Biosafety cabinet with all materials (points from 10 to 16) and turn on UV light at least 30 minutes. At the same time, warm RPMI1640-complete medium in a water bath 37°C
2. ____ Prepare RPMI+CFP adding the CFP at final concentration **50 μg/mL** to RPMI1640-complete medium (Note 1)
3. ____Add the RPMI+CFP (20 mL in T75 flask or 10 mL in T25 flask) to the flask with the adhered cells and incubate at 37 °C with 5% CO_2 for 24 hours (Note 2).
4. ____After incubation, take the supernatant in a sterile 50 mL Falcon and proceed with the exosomes isolation. Discard the cells (Note 3).

Notes:

1. To minimize the risk of contamination in this step, filter RPMI+CFP mix, using a 0.22 μm syringe filter. Instead of CFP, the cells could be infected with different substance-microorganism (see SOP: SP059).
2. As per SOP SP068, wait 72 hours after activating THP1 cells with PMA before adding CFP.
3. Exosome isolation can be performed with centrifugation or using the Exoquick TC (SBI cat # EXOTC50A-1)