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Extracellular Vesicle Group

Protocol for isolating vesicles from cell supernatant

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Verzió szám: 08

Készítette: Pálóczi Krisztina; laboratóriumi analitikus

Angol verzió: Försönits András

Ellenőrizte:

Starting with 30 ml cell supernatant

Reagent, tool

+ filters:
  - 5 µm filter
  - 0,8 µm filter
  - 0,2 µm filter
  - 0,1 µm filter

+ syringes
  - 20 ml
  - 1 ml

+ 25G needles
+ transfer pipett
+ falkon tube
+ eppendorf tubes
+ 15 ml centrifuge tube
+ 10 ml UC tube (thick wall, with its red caps)

+ filtered high glucose [5,5 g/l] AB/AM medium* (from now on, referred to as medium) – it should cointain only the DMEM base medium (1 g/l glucose) suplemented only with antibiotics and glucose: 0,225 g glucose / 50 ml (glucose is 45% Sigma[G8769-100 ml] is in the 4 °C refrigerator, practically you have to add 0,5 ml to 50 ml medium and than filter it through 0,1 µm filter)

  * 1 x PBS or 9 g / 100 ml NaCl can be used as well

+ filtered high glucose PBS
+ if EM is due, than fixer: 4% PFA

  freshly diluted from the 16% stock with PBS or made it on the spot (pH 7,2-7,4)

+ distilled water

+ centrifuges:
  - 8th floor
    - JS 5.3 rotor
    - JA 25.15 rotor
  - 604 Eppendorf / Hermle centrifuge
  - UC: MLA-55 rotor or
  - UC: MLS-50 rotor

Apo

1. Getting rid of cells
   20-30 ml supernatant / tubes (50 ml Falcon)
   2x JS 5.3 / 200 rcf / 5' / RT Accel, Decel: max
2. supernatant into new tubes (pellet can be either recultivated or wasted)
   JS 5.3 / 2.000 rcf / 20' / RT Accel, Decel: slow
3. supernatant into new tubes → MV

4.b getting the pellet into 30-40 ml medium, than filtering it through 5 µm filter. Put the flowthrough into Eppendorf tubes
eppendorf / 2.000 rcf / 20' / RT/ soft

5. sip the supernatant with a needle, and wash the pellets to one (or more if required) tubes. If protein is to be measured of MS or EM measurements are expected, than wash it with PBS, if cells are treated with it, than medium.
   eppendorf / 2.000 rcf / 20' / RT /soft

MV

4.a Filter the supernatant with 5 µm and 0,8 µm filters, put the flowtrough into the 15 ml centrifuge tubes, tare it carefully

JA 25.15/ 12.500 rcf / 20' / 14 °C/ Accel, Decel: slow or
eppendorf / 12.600 rcf / 20' / 16°C/ soft

6. get the supernatant with transfer pipette → exo

7.b wash the pellet into one tube, with medium or PBS

JA 25.15/ 12.500 rcf / 20' / 16 °C/ Accel, Decel: slow *or*
eppendorf */ 12.600 rcf / 20' / 16°C/soft*

Exo

7.a filter the supernatant with 0,2 µm filter, than put it into the UC tubes

MLA-55 / 100.000 rcf / 90' / 4°C / Accel: 8 Decel: 8

8. Mark the spot where the pellet is (or should be)! Get rid of the supernatant with a single move, mop up the drop at the opening of the tube and wash the pellet (the marked area) several times with 100-200 µl buffer
9. Put the sample to 5 ml UC tubes or to 1,8 ml Nunc freezing tubes (both filled to the top so that no harm is done in the tubes) – put the latter into 10 ml UC tubes filled with water

MLS-50 / 100.000 rcf / 60' / 4°C / Accel, Decel: 8 or
MLA-55 / 100.000 rcf / 60' / 4°C / Accel, Decel: 8

10. get rid of the supernatant with a single move (in the case of Nunc tubes, get out 0,5-1 ml liquid from the tube first), get the pellet into desired buffer