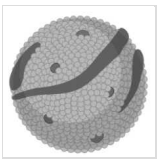

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Nov 25, 2021

2021-11-25 - Plasma Ultracentrifugation Protocol V.2

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protocol .

Dakota Gustafson

Here we describe a bench top protocol for the isolation of extracellular vesicles (EVs) from human plasma. Plasma and other body fluids contain membranous EVs, which are considered to derive from a wide variety of cells. EVs participate in physiological and pathological processes and have potential applications in cell-to-cell communication, diagnostics, and therapeutics. This protocol is intended to isolate a holistic population of EVs using input volumes of at least 1mL. Note, there are a number of nuances for the isolation of 'EVs' including the deep consideration of contaminating/co-precipitating molecules. This protocol is by no means the gold-standard for isolating as density ultracentrifugation will give a prep with lower levels of contaminating lipoproteins. Nonetheless, this protocol does suffice for most applications.

There are a number of articles that may be of interest before you begin:

Brennan K, Martin K, FitzGerald SP, O'Sullivan J, Wu Y, Blanco A, Richardson C, Mc Gee MM (2020). A comparison of methods for the isolation and separation of extracellular vesicles from protein and lipid particles in human serum.. Scientific reports.
https://doi.org/10.1038/s41598-020-57497-7

Crescitelli R, Lässer C, Lötvall J (2021). Isolation and characterization of extracellular vesicle subpopulations from tissues.. Nature protocols.
https://doi.org/10.1038/s41596-020-00466-1

Dakota Gustafson 2021. 2021-11-25 - Plasma Ultracentrifugation Protocol. **protocols.io**
https://protocols.io/view/2021-11-25-plasma-ultracentrifugation-protocol-b2c6qaze
Dakota Gustafson

Extracellular Vesicles, Ultracentrifugation, Human Plasma, Plasma

protocol ,

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- General laboratory safety is described in most institutional biosafety manuals.
- Due to the inherent biological risks of utilizing human samples all sample manipulations should be performed in a BSC located in a CL2 certified facility.
- The use of disinfectant like Virox (or any Accelerated Hydrogen Peroxide equivalent product, like PreEmpt RTU) or bleach when appropriate are the preferred methods of decontamination for experiments utilizing human biospecimens.
- Personal Protective Equipment (PPE) Requirements: Eye protection, closed front medical gown (fluid resistant level II) tied in the back; double gloves (with the outer pair changed frequently inside the BSC after manipulation of samples); procedural mask should be added to prevent sample contamination.

☒ Ice Bucket **Contributed by**

- **users Catalog #M16807-1104**
- ☒ Wet Ice **Contributed by users**
- ☒ Falcon Tube Rack **Contributed by users**

☒ 10mL syringe **VWR international**

- **Ltd Catalog #75846-756**

☒ Protein LoBind Tubes, 1.5

- mL **Eppendorf Catalog #0030108116**

☒ 50ml Falcon

- tubes **Corning Catalog #352070**
- ☒ Gel-Loading Tips, 1-200µL, Volume: 200µL; Length: 2.75 in.; O.D.: 0.6mm; Packaging: BP **Thermo Fisher Catalog #02707181**
- ☒ Pipettes **Contributed by users**

☒ Dulbeccos -/- PBS **Emd**

- **Millipore Catalog #H2OMB0501**

☒ Millex-GP Syringe Filter Unit, 0.22 µm **Emd**

- **Millipore Catalog #SLGP033RS**

☒ 10.4 mL Polycarbonate Bottle with Cap Assembly 16 x 76mm - 6Pk **Beckman**

- **Coulter Catalog #355603**

Ultracentrifugation can be dangerous if the tubes are not properly balanced. Pay close attention to the tube weight when conducting this experiment.

REAGENT AND SAMPLE SETUP 15m

1 Prepare all materials needed for the protocol. 5m

2 Annotate samples and tubes with corresponding sample IDs. 5m

Thaw samples on wet ice for two hours with inversion of tubes every thirty minutes. Sample input should be at least 5m

3 1mL.

4 2m
- While samples are thawing begin purifying the water for sample dilution.
- It's recommended to use a fresh bottle of Dulbecco's $^{-/-}$PBS in combination with the 0.22 µm Millex-GP Syringe Filter Units.
- Draw up 10mL of PBS into a syringe and assemble the filter unit (screw onto the syringe).
- Filter PBS into a 50mL Falcon tube (you need ~14mL of water per sample to be processed).

SAMPLE PREPARATION 1h 5m

5 15m
Once samples are thawed re-centrifuge the samples at **2500 x g, 4°C, 00:15:00** to reduce the remnant platelet and large particle counts.

5.1 15m
Carefully remove the supernatant, leaving ~10uL plasma at the bottom, and transfer to a fresh 1.5mL eppendorf tube.

5.2 15m
Repeat the spin at **2500 x g, 4°C, 00:15:00** to further ensure the clarity and purity of the samples.

6 5m
Carefully remove the supernatant, again leaving ~10uL plasma at the bottom, and transfer to a 10.4 mL, Polycarbonate Bottle.

6.1 5m
- Dilute the plasma sample with 7mL of filtered PBS (total volume of 8mL within the tube; using 1mL input).
- Screw on the caps ensuring a tight seal.

6.2 5m
Using a scale, weight the tubes down to the hundredth decimal to ensure balance while ultracentrifuging at high speeds (weight with caps on).

6.3 5m
Balance tubes down to the hundredth decimal using PBS (write these weights down in your lab notebook).

ULTRACENTRIFUGATION 1h 22m

7 2m
Turn on the Optima XE-90 Ultracentrifuge (Beckman Coulter, Pasadena, CA, USA) with a fixed angle Type 70.1 Ti rotor.

7.1 5m
- Balance the tubes within the fixed angle rotor.
- Apply grease to the interior gaskets (wide outside and central screw) to ensure a tight seal.
- Screw top on and ensure it's tight.
- Place rotor into the ultracentrifuge ensuring no remaining vibration post-placement.

7.2 1h 10m
To run the instrument:
- Ensure the correct tube size is selected in options.
- Calibrate speed to **120000 x g, 4°C, 01:10:00 , k-factor 133.7**

7.3 1m
Start run and wait until instrument gets up to speed before leaving.

SAMPLE WASHING 50m

8 5m
- Once the run is complete carefully remove samples and return to the lab.
- Unscrew the caps and using a vacuum aspirator, aspirate the supernatant. Depending on the input volume and clarity of the plasma a small white pellet should be observable on one axis on the tube wall. Carefully avoid this when aspirating. DO NOT DECANT.

8.1 30m Using a western-blot loading tip (long pipette tip) resuspend the pellet in 200uL of PBS. Take your time, run the sides of the pipette tip along all the edges of the tube to maximize resuspension. It's critical to do this or you can have reduced yield when conducting the final resuspension.

8.2 5m Once the pellet is thoroughly resuspended, add an additional 7.8mL to the tube and screw the cap back on.

8.3 5m Using a scale, weight the tubes down to the hundredth decimal to ensure balance while ultracentrifuging at high speeds (weight with caps on).

8.4 5m Balance tubes down to the hundredth decimal using PBS (write these weights down in your lab notebook).

REPEAT ULTRACENTRIFUGATION

9 1h 10m
- Place samples back into the rotor.
- Start run and wait until instrument gets up to speed before leaving.
- Calibrate speed to **120000 x g, 4°C, 01:10:00 , k-factor 133.7**

RESUSPENSION 41m

10 5m
- Once the run is complete carefully remove samples and return to the lab.
- Unscrew the caps and using a vacuum aspirator, aspirate the supernatant. Depending on the input volume and clarity of the plasma a small white pellet should be observable on one axis on the tube wall. Carefully avoid this when aspirating. DO NOT DECANT.

10.1 30m Using a western-blot loading tip (long pipette tip) resuspend the pellet in 100-200uL of PBS (Volume and resuspension agent are dependent on your downstream applications). Take your time, run the sides of the pipette tip along all the edges of the tube to maximize resuspension. It's critical to do this or you can have reduced yield.

10.2 5m Transfer the supernatant (EV suspension) to a fresh 1.5mL low-bind eppendorf tube.

10.3 1m
- Proceed to downstream applications such as validation experiments or store at -80°C.