Plasma Ultracentrifugation Protocol V.1

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This protocol is intended to isolate a holistic population of extracellular vesicles from human plasma using input volumes of at least 1mL. Note, there are a number of nuances for the isolation of 'extracellular vesicles' 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.

Dakota Gustafson 2021. Plasma Ultracentrifugation Protocol. **protocols.io** https://protocols.io/view/plasma-ultracentrifugation-protocol-b2c2qaye

Extracellular Vesicles, Ultracentrifugation, Human Plasma

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No specific guidelines relating to this protocol.

- Ice Bucket
- Wet Ice
- Falcon Tube Stand (2X)
- 10mL Syringes (5X)
- 1.5mL Low Bind Protein Tubes (Eppendorff, Cat#: 0030108442)
- 50 mL Falcon Tube (Cat#: 352070)
- Western Blot Pipette Tips (200uL)
- Pipette Set
- Dulbecco's $^{-/-}$PBS (Sigma, Cat#: H2OMB0501)
- 0.22 µm Millex-GP Syringe Filter Unit (Millipore Sigma, Cat#: SLGP033RS)
- 10.4 mL, Polycarbonate Bottle with Cap Assembly, 16 x 76mm - 6Pk (Beckman, Cat#: 355603)

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

Sample Thawing and Set Up 15m

1 Prepare all materials needed for the protocol, including an ice bucket with wet ice. 5m

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

3 Thaw samples on ice for two hours with inversion of tubes every thirty minutes. Sample input should be at least 1mL. 5m

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 Once samples are thawed re-centrifuge the samples at 2,500xg, 4°C, for 15 minutes to reduce the remnant platelet and large particle counts. 15m

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

5.2 Repeat the spin at 2,500xg, 4°C, for 15 minutes to further ensure the clarity and purity of the samples. 15m

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

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

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

Ultracentrifugation 1h 22m

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

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 To run the instrument: 1h 15m
- Ensure the correct tube size is selected in options.
- Calibrate speed to 92,000×g_{avg} running at 4°C (k-factor: 133.7).
- Set time of run to 70 minutes.

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

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).

Ultracentrifugation

9 1h 15m
- Place samples back into the rotor.
- Start run and wait until instrument gets up to speed before leaving.

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