Plasma Exosome Endothelial Inflammation Kit

Appendix 1 - Platelet Poor Plasma (PPP) Preparation

1. PREPARATION OF PPP COLLECTION BUFFER

Equipment & Consumables:

Equipment Name	Manufacturer & Catalog Number	Product Link
100 µl serological pipettes	Fisher Scientific 50-202-113	Serological pipettes
15 ml conical tubes	ThermoFisher Scientific 339650	15ml tube
Sterile microcentrifuge tubes		
Tube rack		
BD Vacutainer Glass Blood Collection Tube with ACD, 6 ml	Fisher Scientific 02-684-29	BD Vacutainer 364816
Eppendorf Research Plus Pipette: 1-10 µl,10-100 µl, 200 µl, 1000 µl (calibrated <6months)		
Tube Revolver 120V/US PLUG, speed set to 10 rpm	Fisher Scientific 11676341	Tube revolver
Plate/tube shaker		
Benchtop cooling centrifuge		
Vortex		
VersaMax ELISA (Absorbance Microplate Reader): Molecular Devices		
Ice Tray/ Bucket		
Sterile Flasks		

Product Name	Manufacturer & Catalog Number	Product Link
Dulbecco's Balanced Salt Solution (DPBS) no calcium or magnesium, 1,000 ml	ThermoFisher 14190-136	Gibco DPBS
Prostaglandin E1 (PGE1)	Sigma-Aldrich/ MilliPore-SIGMA P5515	PGE1
EDTA (0.5M), pH 8.0, RNase-free	ThermoFisher AM9260G OR AMG9269G	EDTA
Acetone	Sigma-Aldrich/ MilliPore-SIGMA 650501-1L	Acetone
0.1 M Phosphate Buffer Solution	Sigma-Aldrich/ MilliPore-SIGMA P5244-100mL	Phosphate Buffer

Reconstitution of PGE1 (5mg):

1. Spin down the vial (max spin for 30 sec in a benchtop centrifuge).
2. Reconstitute PGE1 by adding 500 µl acetone to the vial and slowly pipet up and down to dissolve the lyophilized PGE1 powder to get a 10 mg/ml solution.
3. Then add 500 µl of 0.1M phosphate buffer to get a stock solution of 5mg/ml.
4. Aliquot 90 µl of PGE1 to sterile microcentrifuge tubes (total 11) and store at -80˚C until further use.

To prepare a PPP collection buffer solution of 600ml: DBS with 2 mM EDTA and 2 µM PGE1

1. Using a combination of the 100 ml serological pipette and smaller pipettes, aliquot 597.515ml DBS (5x 100 mL + 1x 97 ml + 1x 515 µl) into a sterile (autoclaved) flask.

2. Add 2.4 ml EDTA (0.5M) to the DBS in the flask and mix.

3. Add 85 µl of the PGE1 (5mg/ml) to the DBS in the flask and mix.

4. Aliquot 3 ml of buffer (DBS with 2 mM EDTA and 2 µM PGE1) into 15 ml conical tubes and store them at -80˚C.

2. PREPARATION OF PPP FROM BLOOD:

1. 6 ml of venous blood is drawn (per MarkVCID's Best Practices and Fluid Biosample Requirements) into a tube containing acid citrate dextrose (ACD) anticoagulant solution.

2. Centrifuge the tubes at 500xg for 20 min at room temperature.

3. Thaw out the required number of tubes containing PPP collection buffer.

4. Using a 1 ml pipette, gently transfer 3 ml (3 x 1 ml) of platelet-rich plasma (PRP) to the 15 ml falcon tubes containing the thawed pre-aliquoted PPP collection buffer. **When removing the PRP, it is essential not to disturb the pellet at the bottom of the ACD plasma tube.**

5. Pipet up and down to mix the PRP with the collection buffer.

6. Centrifuge the PRP and buffer solution at 2,200xg for 20 min at room temperature.

7. Then, 0.25 ml aliquots of PPP are transferred to 1.5 ml Eppendorf tubes and stored at -80^{o}C.

Questions/Comments?
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