

Isolation of Plasma – Undiluted, Pre-Ficoll (EDTA)

Equipment:

- Benchtop centrifuge (Allegra X-15R, Beckman Coulter)
- Pipette Gun (Drummond)

Materials:

- EDTA Lavender Top Tube (Fisher, #367861)
- 1.8mL Cryotube vials (Fisher, #375418)
- 15mL conical vial (Fisher, #1495949B)
- 2mL, sterile, serological pipettes (Fisher, #356507)

Reagents:

- Ca⁺ and Mg⁺ Free PBS (Invitrogen, 10010-049)

Procedure:

1. Collect whole blood from subject into EDTA Lavendar Top Tube
2. Centrifuge collection tubes tubes at 200 x g for 10 minutes at RT.
3. Carefully remove collection tubes from centrifuge; plasma is the top layer above the rest of the whole blood layer.
4. Using caution not bring up the remaining whole blood layer, remove 2.5mL of plasma into a 15mL conical vial using a pipette gun and a 2mL sterile, serological pipette.
 - a. If continuing on to isolate PBMCs, replace the plasma removed with an equal amount of PBS in the tubes.
5. Centrifuge the isolated plasma at 1,000 x g for 10 minutes.
6. Aliquot the plasma supernatant (refer to Appendix A for aliquot quantities and volumes) into tubes labeled with appropriate de-identified cryogenic labels.
7. Freeze at -80°C.

Appendix A – HIMC Aliquot Guidelines

Plasma***
(Pre-ficoll Whole Blood)

Total Plasma Volume	Standard Volume/Aliquot	Required # of vials	Minimum Volume per aliquot	Maximum Volume per aliquot
up to 3ccs	120uL	4	120uL	200uL
3-5ccs	200uL	4	120uL	200uL
> 5ccs	350uL	4	200uL	200uL

***Plasma should be processed according to HIMC SOP. Harvest plasma prior to Ficoll and before diluting whole blood. Provide a second spin on isolated plasma prior to aliquotting.

***Flu Studies: Additional aliquots of plasma may be required for other collaborators. Refer to Flu Distribution chart for those details and volume requirements.