Ritchlin Laboratory

PBMC and Monocyte Isolation from Whole Blood

Date: 11JANUARY2018

1.0 Subject
Isolate PBMCs and Monocytes from Whole blood of Healthy donors and/or Patients.

2.0 Purpose
Cells isolated will be used for various experiments (i.e. Flow Cytometry, Culture) and stored in frozen storage (-80).

3.0 Scope
The scope of this procedure applies to the R01 Grant provided by the NIH for BSL-2 operations in the Ritchlin laboratory.

4.0 Safety Concerns
Working with patient blood and sera are considered BSL-2.

5.0 Definitions

6.0 Materials

6.1 Vacutainer Tubes
1. Green Top- Heparin, PBMC/Monocyte
2. Red Top- Serum
3. Blue Top- Tempus, RNA
4. Lavender- DNA

6.2 Blood Processing
1. 1X PBS (Corning #21-040-CV)
2. 0.5M EDTA (Invitrogen #AM9260G)
3. Ficoll-Plaque Plus (GE #17-1440-03)
4. ACK Lysis buffer (Gibco #A10492-01)
5. Human Monocyte Enrichment Cocktail (Stemcell-Rosettsep #15068)
6. SepMate, 50mL Tube (Stemcell #85450)
7. 50ml and 15ml Conical tubes
8. 1.5ml mini-centrifuge
9. Polypropylene 5ml flow tube
10. Polypropylene 5ml flow tube with cell strainer (#352235)

7.0 Specimens

7.1 Human Cells stored within freezer vials with DMSO and freeze media.

8.0 Procedure

8.1 Blood from green top vacutainer tubes are divided into 50ml Conical tubes, max 18mls/tubes.

8.2 **FOR MONOCYTES**: Human Enrichment cocktail is added to the tubes labeled monocytes, 50uL/mL and EDTA 0.5M at a final concentration of 1mM. Let sit RT for 20min.

8.2.1 18mls blood= 400uL Enrichment Cocktail, 50uL EDTA.

8.3 Serum/Plasma Collection:

8.3.1 Label four 1.5ml centrifuge tubes. 1200uL of blood from **PBMC** tubes is added to each and spun. 4500rpm for 10min, RT (for Plasma).

8.3.2 Spin Red top (For serum) vacutainer, 2300rpm for 10min for 10min.

8.3.3 Aliquot 600uL plasma into pre-labeled plasma tubes. Aliquot 600ul of serum from red top tube into pre-labeled serum tubes.

8.3.4 Store in -80°C Freezer.

8.4 Ficoll Gradient:

8.4.1 Traditional method:

8.4.1.1 Add 20mls of Ficoll to new 50mL conical replicating number of blood tubes. E.x. 2 blood tubes, 2 Ficoll tubes.

8.4.1.2 Add up to 30mls 1X PBS to whole blood tubes.

8.4.1.3 Tilt Ficoll tube and pipette diluted blood SLOWLY OVER Ficoll, not to mix the two.

8.4.1.4 Spin 2350rpm for 30min, no brake.

8.4.1.5 CAREFULLY remove intermediate phase (buffy coat) and transfer to new 50mL conical tube.

8.4.1.6 Add 1XPBS up to 40mls. Spin 1400rpm, 10 min.

8.4.2 Sepmate Method:

8.4.2.1 Add 15mls of Ficoll through bottom opening of SepMate lower chamber, no not blow bubbles into opening. Some Ficoll will remain outside of lower chamber.

8.4.2.2 Add up to 30mls 1X PBS to whole blood tubes.

8.4.2.3 Slowly pour/pipette diluted blood over Ficoll filled chamber.

8.4.2.4 Spin 1200xg for 10 min.

8.4.2.5 Pour contents of Sepmate into new 50mL conical tube by quick inverting, no longer than 2 seconds.

8.4.2.6 Add 1XPBS up to 50mls. Spin 1400rpm, 10 min.

8.5 Cell preparation:

8.5.1 Cells are re-suspended 1mL of ACK lysis buffer, 6min room temperature.

8.5.2 Washed with 1X PBS, spun 1400rpm 10min.

8.5.3 Cells re-suspended in 4mls of 1XPBS and transferred to a labeled 5ml flow tube with cell strainer.

8.5.4 Count cells: 1:10, 45ul Trypan Blue + 5ul Cells.

Date	ID#	Cell	Count(M/mL)	0.5M(ul)	1.5M(uL)	1.0M(uL)	0.1M(uL)	0.2M(uL)	0.1Mx2(uL)	0.2Mx2(uL)	2M(uL)
Date	Patient ID	PBMC	5	100	300	200	20	40	40	80	400
	Blood	Mono	2	250	750	500	50	100	100	200	

8.5.5 $1.5x10^6$cells are used for staining.

8.5.6 $0.1x10^6$ and $0.2x10^6$cells are used for OCP culture in duplicate.

8.5.7 5ml polystyrene flow tubes are labeled as followed:

8.5.7.1 PBMCs

8.5.7.2 Monocytes

8.5.7.3 Unstained

8.5.7.4 P1 ($0.1x10^6$ PBMC)

8.5.7.5 P2 ($0.2x10^6$ PBMC)

8.5.7.6 M1 ($0.1x10^6$ Monocytes)

8.5.7.7 M2 ($0.2x10^6$ Monocytes)

8.5.8 Add 2mls of 1X PBS to all flow tubes and 2mls of CM media to OCP tubes. Spin 1400RPM, 5min RT.

8.5.8.1 CM media:

8.5.8.1.1 Alpha MEM, 10% FBS, 5% pen/strep

8.5.9 Continue on for cell staining and OCP culture.

8.6 Cell Storage

8.6.1 Spin cell strainer tubes down

8.6.1.1 1400 RPM, 5 min RT

8.6.2 Label cryovials with BSI labels.

8.6.3 Add 200ul each vial with both Freeze media A and B

8.6.4 Distribute cells evenly to each vial.