SOP: Isolation of Immune cells by Stemcell Technologies Magnetic Beads kits and subsequent activation or stimulation
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The following protocol describes the isolation of various immune cell fractions from PBMC's isolated from full leukopacks. These cell fractions were subsequently activated or stimulated, as appropriate to the cell types and reagents used.

Reagent Ordering Information

Item	Catalog Number	Manufacturer
Leukopack	various	Allcells or StemExpress
Ficoll	17144003	Sigma-Aldrich
PBS	10010023	ThermoFisher
Isolation kits	See pg. 4	Stemcell Technologies
EasySep buffer	20144	Stemcell Technologies
RPMI 1640 Media	11-875-135	Fisher Scientific
FBS	SH3007103	Fisher Scientific
Pen/Strep	SV30010	Fisher Scientific
Human IL-2	200-02	Peprotech
Human IL-4	200-04	Peprotech
Human IL-12	200-12H	Peprotech
Human IL-15	200-15	Peprotech
Human IL-18	9124-IL-050	R&D Systems
Anti-IgM Fab'(2)	16-5099-85	ThermoFisher
Anti-CD40	334302	Biolegend
Dynabeads Human T-Activator CD3/CD28	11132D	ThermoFisher

Materials List

Item	Catalog Number	Manufacturer
Easy Sep magnet	18000	Stemcell Technologies

PBMC isolation

Isolating PBMC's from leukopak (sources: Allcells or StemExpress)

1. In the hood, transfer Leukapheresis product into 2x 50ml conical. Original tube contained ~49mL blood product.

2. Slowly dilute Leukapheresis product with 20ml room temperature PBS.

3. Transfer half of the volume into a second 50ml conical and add PBS to bring the volume in both tubes up to 35ml.

4. Pipette 12 ml of Ficoll (Sigma-Aldrich, cat# 17144003) into two new 50ml conicals.

5. Carefully (using "slow" settings on pipette-aid) layer 35ml diluted Leukapheresis product over the Ficoll. To obtain good separations, it is critical that a clear interface be kept between the Ficoll and the blood layers before centrifugation.

6. Spin at 830xg for 20min, at 20°C, no brake in a swinging bucket rotor.

7. Carefully transfer the tubes from the centrifuge without disturbing the layering. Due to red clouding/opaque nature of the top layer, entire top layer above ficoll was removed, resuspended up to 35mL with PBS, and re-layered on new ficoll and re-spun using the above conditions.

8. Carefully aspirate the PBMC layer (white Buffy coat) from the tube using a 5ml pipette on "slow" and transfer to a new 50ml tube. Avoid aspirating Ficoll. Discard the remainder of the Ficoll and red blood cells.

9. Wash PBMC by adding room temperature PBS to 45ml.

10. Spin at 430xg for 10 min, with normal deceleration (break on).

11. Decant the supernatant, loosen pellet and wash once more in 45ml RT PBS +2% FBS.

12. Spin at 430xg for 5 min. Decant the supernatant, loosen pellet and resuspend the cells in 8ml EasySep Buffer (PBS with 2% FBS and 1mM EDTA).

13. Count the cells with a hemocytometer or automatic counter and determine the cell count and viability with Trypan Blue. (Perform a 1:50 dilution for counting, or adjust as needed)

14. At this point cells can either be viably frozen at 100M cells/ml in RPMI with 50% FBS and 10% DMSO, or they can be used fresh for isolation using Stemcell Technology's bead kits.

Stemcell Technology bead kits used for cell isolations:

Cells are isolated according to manufacturer's instructions

Pan T-cells (CD3+): cat # 17951

Naïve CD4+ T cells: cat# 19554

Th17 (CD4+) T cells: cat# 17862

Treg (CD4+) T cells: cat# 19232

Central Memory CD8+ T cells: cat# 17869

Effector Memory CD8+ T cells: cat# 17869

NK cells: cat# 19055

Naïve B cells and Memory B cells: First isolate pan B cells (cat# 17954) and then isolate naïve and memory B cells (cat # 17864)

Cell stimulation protocols and reagents:

Base Media: RPMI with 10%FBS, 1% PenStrep, Stimulation time = 72 hours
antiCD3/CD28 beads are used at a 1:1 ratio to cell number

Pan T-cells (CD3+):
Baseline t = 0
Activation: 50U/ml IL-2 and antiCD3/CD28 beads

Naïve CD4+ T cells:
Baseline t = 0
Activation: 50U/ml IL-2 and antiCD3/CD28 beads

Th17 (CD4+) T cells:
Baseline t = 0
Activation: 50U/ml IL-2 and antiCD3/CD28 beads

Treg (CD4+) T cells:
Baseline t = 0
Activation: 50U/ml IL-2 and antiCD3/CD28 beads

CD8 Naïve T cells:
Baseline t = 0
Activation: 50U/ml IL-2 and antiCD3/CD28 beads
Activation with Stimulation: 50U/ml IL-2, 100ng/ml IL-15 and antiCD3/CD28 beads

Central Memory CD8+ T cells:
Baseline t = 0
Activation: 50U/ml IL-2 and antiCD3/CD28 beads
Activation with Stimulation: 50U/ml IL-2, 100ng/ml IL-15 and antiCD3/CD28 beads

Effector Memory CD8+ T cells:
Baseline t = 0
Activation: 50U/ml IL-2 and antiCD3/CD28 beads
Activation with Stimulation: 50U/ml IL-2, 100ng/ml IL-12 and antiCD3/CD28 beads

NK cells:
Baseline t = 0
Stimulation: 100ng/ml IL-12, 100ng/ml IL-15, 100ng/ml IL-18

B cells:
Baseline t = 0
Stimulation: 100uM CD40 +100ng/ml IL-4
Activation with Stimulation: BCR activation using 10ug/ml anti-IgM Fab'(2)) + 1 ug/ml CD40 + 100ng/ml IL-4