Protocol: PBMC Freezing and Thawing with LN2

Purpose: Freezing and thawing peripheral blood mononuclear cells using liquid nitrogen. Can use either 90% FBS Freezing Medium or 40% FBS Freezing Medium - please see Reagents below.

Conditions: *All steps conducted under sterile conditions for cell culture.*

Scale: 10E6 PBMCs/cryovial

Materials:
2 mL screw cap micro tube (Sarstedt #72.694.306) (RT)
15 mL conical tube (Sarstedt #62.554.205) (RT)
Countess Automated Cell Counter (or hemocytometer)
Countess cell counting chamber slides (Invitrogen #C10283) (RT)
Trypan blue stain 0.4% (Invitrogen #T10282) (RT)
RPMI-1640 (ATCC #30-2001) (4℃)
FBS (Thermofisher #A5256801) (4℃)
DMSO (Sigma #D2650-100ML) (RT)
Pen/strep (Gibco #15140-122) (4℃)
Mr. Frosty cryocontainer filled with isopropanol (4℃)
DNase I (25 MU) (Millipore Sigma #260913-25MU) (-20℃)
Water (Molecular biology-grade) (Quality Biological #351-029-131) (RT)

Reagents:

90% FBS Freezing Medium (4℃) (125mL)
+ 112.5 mL FBS (90% final)
+ 12.5 mL DMSO (10% final)
+ Sterile filter with 0.22 uM

Full RPMI Media (4℃) (500 mL)
+ 450 mL RPMI-1640 (90% final)
+ 50 mL FBS (10% final)
+ 5 mL pen-strep (1% final)
+ Sterile filter with 0.22 uM

40% FBS Freezing Medium (4℃) (125 mL)
+ 62.5 mL RPMI-1640 (50% final)
+ 50 mL FBS (40% final)
+ 12.5 mL DMSO (10% final)
+ Sterile filter with 0.22 uM

DNase final conc. 1000 U/ul (-20℃) (25 mL)
+ Resuspend pellet in 25 mL sterile H20
+ Sterile filter with 0.22 uM syringe filter
+ Aliquot 100 ul/tube into PCR strip tubes

Freezing PBMCs for LN2 storage

1. Quantify cells using automated cell counter or hemocytometer. In a 96-well plate or 1.5 mL e-tube, thoroughly mix 10 ul of cell stock and 10 ul of Trypan Blue 0.4%. Transfer 10 ul of this mixture to the chamber slide and acquire quantification. Freeze PBMCs at 10E6 PBMC/vial (e.g. if there are 50E6 PBMCs, there will be (5) 10E6 PBMC/vial freezedowns).

2. Once quantified, spin cell suspension at 400xg for 5 mins at 4℃ (9 acceleration/9 deceleration). While spinning, begin labeling the number of cryovials needed for freeze down.

3. After spinning, resuspend cell pellet in the calculated volume of ice-cold freezing media, accordingly (e.g. if there are 50E6 PBMCs in the pellet, resuspend in 5 mL freeze media, for 10E6 cells/mL).

4. Immediately aliquot 1 mL of cells (10E6 PBMCs) into each cryovial and transfer to a pre-chilled Mr. Frosty and store at -80℃ overnight.

5. After 24 hours, transfer cryovials from Mr. Frosty to LN2 storage.

Thawing PBMCs for analysis/cell culture (PBMCs do not expand in cell culture)

1. Before thawing (1) freeze down, add pre-warmed 9 mL Full RPMI media (37°C) into 15 mL conical. Add 10 ul of DNase (1000 U/ul) to the media (for a final concentration of 1 U DNase/ul) to avoid cell clumping/damage.

2. Nearly thaw (1) cryovial in a 37°C water or bead bath (about 2 mins). Do not allow the sample to reach 37°C/warm as the DMSO will damage the cells. Add the 1 mL of thawed cells directly to the 15 mL conical with full RPMI media + DNase.

3. Spin cell suspension at 400xg for 5 mins at 4°C (9 acceleration/9 deceleration). While spinning, prepare the next vessel (i.e. flow tubes if doing flow analysis or suspension T75s if doing cell culture).

4. Resuspend pellet in 10 mL full RPMI media for counting/analyses/maintenance.