

LABORATORY PROCEDURE: Freeze/Thaw PBMC

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PURPOSE: To properly freeze cells and thaw cells for the maximum return yield

SCOPE: This procedure applies to all cells including PBMC, BM and cell lines

PRINCIPLE: Cells are frozen in a cryoprotectant and slowly frozen at -1C per hour for 24 hours. Cells are thawed quickly using 37C media and a 37C water bath and quickly diluted to reduce the concentration of the cryoprotectant.

SAFETY PRECAUTIONS: All work should be performed under the biological safety cabinet observing safety regulations and using sterile technique. Personal protective equipment such as: lab coat, gloves and glasses, should be used during the procedure. Specimens should be handles as if capable of transmitting infection. All contaminated supplies should be properly disposed of in biohazard or sharps containers and liquid waste should be decontaminated with bleach for 20min before being poured down the drain.

NOTE: Pay particular attention to “**HOT SPOT**” steps. These are crucial to optimize cell yield and viability.

MATERIALS AND REAGENTS:

**Supplies**

100um filter (*VWR 89508-340*)  
2ml cryovials (*VWR 89094-810*)  
15ml conical (*Falcon 352097*)  
50ml conical (*Falcon 352070*)  
10 ml pipet (*VWR 89130-898*)  
Dry ice  
Cryolabels

**Equipment**

37C water bath  
Centrifuge  
Pipet aid  
Waste container for liquid  
Freeze jar (*Bioscission- Cool Cell*)  
-80C freezer  
-196C freezer  
P1000+tip  
Bucket

**Reagents**

RPMI complete  
Freeze media  
RPMI 1640 (*Invitrogen 11875-093*)  
HI-FBS (*Atlanta Biologicals*)  
Pen/Strep (*Invitrogen 15140-122*)  
70% ETOH  
DMSO (*Sigma- 472301*)

## Freeze/Thaw PBMC

REAGENT PREPARATION: a. Freezing Media: 45ml of HI-FBS run over a 100um filter in a 50ml conical. Add 5 ml of DMSO and invert to mix. b. RPMI complete: to a 500ml RPMI 1640 bottle add 50 ml of HI-FBS and 5ml of Pen/Strep. Invert to mix.

REAGENT STORAGE: **Room Temperature**: DMSO, 70% ETOH **4C**: Freeze media, RPMI1640, RPMI complete, HI-FBS. **-20C**: Pen/Strep

SPECIMAN STORAGE: When freezing cells, remove from 4C or ice and aliquot as quickly as possible. When thawing cells vials should remain on dry ice until ready to thaw.

QUALITY CONTROL: Ensure that the freeze media is clear in color (not cloudy)

### PROCEDURE:

#### **FREEZE:**

- After counting the cells (see counting SOP), determine how many cryovials you will need to freeze approximately 10 million per vial. Label vials (cryolabel for -196C, or a permanent lab marker) with Patient ID-RSRB-visit#, cells #, and date.  
Example 00027-49798-v01, 10 mill, 03-10-15
- Centrifuge at 350 RCF/g for 6 minutes
- Invert to discard liquid in waste container
- Resuspend the cells in 1ml of CryoStor
- Add the remaining volume of Cryostor and thoroughly resuspend cells in full volume
- Utilizing a serological pipet or a p1000 aliquot 1ml of the cells into the cryovials
- Place vials in freeze jar and place at -80C for minimum of 24 hours
- Move cells for long term storage to -196C

#### **THAW:** **^HOT SPOT** Pre-warm RPMI complete in 37C waterbath

- Remove cells from the freezer and place on Dry ice
- Place 5ml of warm RPMI complete in 15ml conical per patient (not per vial)
- Thaw no more than 3 vials at a time/per 15ml conical. Place 1ml of warm RPMI complete into the vial and submerge in a 37C water bath. Vials **MUST** be monitored at all times. **^HOT SPOT** at short intervals check the vials and invert. At the precise time it is thawed, spray with 70% ETOH and transfer cells to your labeled 15 ml conical containing the 5ml of media with a p1000.

- Centrifuge at 350 RCF/g for 6 minutes
- Invert to discard liquid in waste container
- Resuspend in necessary media/volume for your assay
- Count cells on hemocytometer (see counting SOP)

LIMITATIONS: No limitations

CALCULATIONS: Freezing: total cell number divided by  $10 \times 10^6 = \#$  of vials. Thawing: Calculations can be obtained in the counting SOP

INTERPRETATION: When thawing cells, after resuspension cells may clump. These are dead cells and that can be removed before counting. We anticipate up to 50% loss when thawing. A large conglomerate of dead cells is an indicator of poor quality cells when freezing or too long in 37C water bath when thawing.

RESULTS REPORTING: The results for freezing cells are reported with Sample ID, Date, total cell# frozen and number of vials frozen. The results for thawing cells are reported as # of vials thawed, cell per vial at time of freezing and total cell # after thaw.

TRAINING: Personnel will be trained by staff. Up to one time visual shadowing of staff member AND up to one time hands on training with staff member AND one or more times independent performance with successful completion of SOP.