CATEGORY: Laboratory Procedures	**SOP Number**: Version 002
TITLE: Method for thawing frozen cells	

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Number	**Section**	**Pages**	**Initials/Dates**

1.0	**Title**
	Method For Thawing Frozen Cells

2.0	**Purpose**
	To describe the procedure for thawing frozen cells that have been preserved on liquid nitrogen

3.0	**Definitions and Abbreviations**	
	PBMC	Peripheral Blood Mononuclear Cells
	NEAA	Non Essential Amino Acids

4.0 Equipment, Materials, Reagents

4.1 Equipment

		Title	**Vendor**	**Catalog #**
4.1.1	Centrifuge Dupont Rotor H1000B	Sorvall	T6000D H1000B
4.1.2	14 ml Conical Tubes Rotor or 50 ml Conical Tubes Rotor	Sorvall	H1000B PN11093
4.1.3	Conical Tubes, 50ml, sterile	Falcon Fisher	352074 14-432-22
4.1.4	Sterile Conical Tubes, 15ml, sterile	Falcon	14-959-70C
4.1.5	Dry ice and dry ice containers		
4.2.6	Ice buckets		
4.1.7	37^{o}C, water bath		
4.1.8	Serological pipette, 5 ml	Corning VWR	4051 29442-422

			10ml	Corning VWR	4101 29442-430
		4.1.9	Pasteur pipette		
		4.1.10	Vacuum device		
		4.1.11	Pipettor		
	4.2	Reagents			
			Title	**Vendor**	**Catalog #**
		4.2.1	Frozen PBMCs	Subject Source	
		4.2.2	Tissue culture tested, heat inactivated, filtered, HuAB serum	ITN serum Lot	H90911V
		4.2.3	HL-1 medium	Fisher	77201
		4.2.4	L-Glutamine	Biowhittaker	17-605E
		4.2.5	Na Pyruvate	Gibco	11360-070
		4.2.6	NEAA	Gibco	11140-050
		4.2.7	Hepes	Biowhittaker	17-737E
		4.2.8	Penicillin/Streptomycin	Biowhittaker	17-602E

Note :	To maintain the membrane lipid fluidity of the cells **do not work with chilled reagents**. Keep all reagents at room temperature.		
5.0	**Procedures**		
	All work needs to be performed under the biological safety cabinet observing bio-safety regulations for BL2 level and using sterile technique.		
	5.1.	Thawing of cells	
		5.1.1	Warm the necessary volume of both complete medium + 10% human AB serum and complete medium+ 5% human AB serum to room temperature. See appendix A for complete medium preparation.
		5.1.2	Contain an aliquot of complete medium + 10% human AB serum

			(formula: 4 times frozen cell volume) in a 15 or 50 ml conical tube Use 15 ml conical tube if total volume of frozen sample from all criovials is up to 3 ml. Use 50 ml conical tube if total volume of frozen sample is more than 3 ml.
		5.1.3	Remove the cryotubes from liquid nitrogen and transfer them to the culture room on dry ice.
		5.1.4	Place the cryovials in the tube holder and thaw the cells quickly in a 37^{o}C water bath or 37^{o}C incubator. Keep the cells at 37^{o}C until they have just begun to thaw and there is a solid frozen core surrounded by liquid. This step will take approximately, 3- 4 minutes per milliliter of frozen cells. Continuously monitor the cell condition.
		5.1.5	Rapidly transfer the contents of the cryovials by pouring it into a prepared 50 or 15 ml conical tube. Rinse each vial with 1 ml of complete medium + 10% human AB serum (use medium from same conical tube).
		5.1.6	Mix the cells gently by pipetting 3 times.
		5.1.7	Immediately, centrifuge the cell suspension at 1200 rpm for 10 minutes.
		5.1.8	Remove the supernatant with a Pasteur pipette connected to a vacuum device. Avoid getting the pipette close to the pellet.
		5.1.9	Tap the tube gently to re-suspend the cell pellet and then reconstitute the pellet in 25 ml of complete medium+ 5% human AB serum.
		5.1.10	Wash the cells by centrifugation at 1200 rpm for 10 minutes.
		5.1.11	Remove the supernatant. as described in 5.1.8
		5.1.12	Re-suspend the pellet in an adequate volume of complete medium + 5% human AB serum. For example: If you are starting with 1 vial containing 1 ml of frozen cells, re-suspend the cells in 2.5 ml of medium; 2 vials of 1 ml each should be re-suspended in 5 ml. Adjust the re-suspension volume proportionally to the original frozen volume that you started with.
		5.1.11	Mix the cells gently. Remove a 20 µl aliquot for cell counting.
		5.1.12	Count the cells. (Use SOP for cell count.)
		5.1.13	The cells are ready for the assay.

Appendix A

Complete medium:

500 ml sterile HL-1 medium (one bottle)

5 ml L-Glutamine (vortex before you add to re-suspend the precipitate)

5 ml Penicilline/Streptomycin

5 ml NEAA

5 ml HEPES

5 ml Sodium pyruvate

Invert to mix. Label it with the name (complete medium) and the date and store at 4^{o}C. Medium may be kept for up to 3 weeks.