# STANDARD OPERATING PROCEDURE

| Title: Thawing of Frozen Cells | SOP No.: | Version: 1 |
|---|---|---|
| | Effective Date: 9/27/2011 | Page: **1 of 5** |
| Trial Number (if applicable): | | |

## Document Approval

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| **Prepared by**: Michael Konieczny | **Signed:** |
| | **Title:** Assay Analyst |
| | **Date:** 9/27/2011 |

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| **Approved by**: Mary Carmelle Philogene | **Signed:** |
| | **Title:** Associate Director Assay Development and Quality Assurance |
| | **Date:** 10/3/2011 |

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## 1 Purpose

To describe the procedure for thawing frozen cells that have been preserved in liquid nitrogen

## 2 Departments Affected

Tolerance Assay and Data Analysis

Rutgers University Central Cell Isolation and DNA Repository

## 3 Definitions and Abbreviations

PBMC – Peripheral Blood Mononuclear Cells

NEAA – Non Essential Amino Acids

RUCDR – Rutgers University Central Cell Isolation and DNA Repository

## 4 Equipment and Materials

**Equipment**

Centrifuge, Dupont Rotor H1000B

14 mL or 50 mL Conical Tubes Rotor

Dry ice and dry ice containers

Ice buckets

Water bath, heated to 37° C

Serological pipette, 5 mL, 10 mL

Pasteur pipette

Vacuum device

Pipettor

**Reagents**

Frozen PBMCs

Tissue culture tested, heat inactivated, filtered, normal human serum AB

HL-1 medium

L-glutamine

Na Pyruvate

NEAA

Hepes

Penicillin / Streptomycin

## 5 Procedure

All work needs to be performed under a biological safety cabinet observing bio-safety regulations for BL2 level using sterile technique

**Thawing of Cells**

5.1 Warm necessary volume of both 'complete medium + 10% human AB serum' and 'complete medium +5% human AB serum' to room temperature. (see below for media preparation

*Complete medium*: (used for washing and re-suspension)

500 mL sterile HL-1 medium (one bottle)
5 mL L-glutamine (vortex before adding to re-suspend the precipitate)
5 mL Penicillin/Streptomycin
5 mL NEAA
5 mL Hepes
5 mL Sodium pyruvate

Invert to mix. Label with name (complete medium) and the date and store at 4 degrees Celsius. Medium may be kept for up to 3 weeks.

*Complete medium + 10% human AB serum*

To prepare 500 mL of complete medium + 10% human AB serum, remove 50 mL of the media from the vial, aliquot 50 mL of the human AB serum, and add to the 450 mL of media. This mix will be used at 5.5.

*Complete medium + 5% human AB serum*

To prepare 500 mL of complete medium + 5% human AB serum, remove 25 mL of the media from the vial, aliquot 25 mL of human AB serum, and add to the 475 mL of media. This mix will be used at 5.9 and 5.12.

5.2 Aliquot the appropriate volume of 'complete medium +10% human AB serum' a conical tube. IMPORTANT: Volume of media should be 4 times amount of frozen cells (Example: for 1mL of frozen cells, use 4mL of media)

a. Use 15 mL conical tube if total volume of frozen sample is up to 3 mL
b. Use 50 mL conical tube if total volume of frozen sample is greater than 3 mL

5.3 Remove the cryovials from liquid nitrogen and transfer them to the culture room on dry ice

5.4 Place cryovials in the tube holder and thaw cells quickly in a water bath at 37° Celsius (or an incubator at 37° Celsius). Keep the cells at 37° Celsius until they have just begun to thaw and there is a solid frozen core surrounded by liquid. This step will take approximately 3 to 4 minutes per milliliter of frozen cells. Carefully monitor the cell condition.

5.5 Rapidly transfer the contents of the cryovials by pouring into the prepared conical tube Rinse each vial emptied with 1 mL of 'complete medium +10% human AB serum'. Use medium from same conical tube.

5.6 Mix cells "gently" by pipetting 3 times. NOTE: Gentle pipetting is important to avoid damaging cells.

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5.7 Immediately centrifuge the cell suspension at 1200 rpm for 7 to 10 minutes

5.8 Remove the supernatant with a Pasteur pipette connected to a vacuum device. Avoid bringing the tip of the pipette close to the pellet. Leave ~200 uL of media on the cell pellet.

5.9 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'.

5.10 Wash the cells by centrifugation at 1200 rpm for7-10 minutes

5.11 Remove the supernatant again, as described in 5.8. Leave ~200 uL of media on the cell pellet.

5.12 Re-suspend the pellet in an adequate volume of 'complete medium +5% human AB serum'.

> For example: If 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; etc. Adjust the re-suspension volume proportionally to the original frozen volume total.

5.13 Mix cells gently. Remove a 20 uL aliquot for cell counting.