STANDARD OPERATING PROCEDURE				
Ragon Institute of MGH, MIT and Harvard	Thawing Frozen Cells		**SOP#**	TC-SOP-003
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I. PURPOSE

The purpose of this procedure is to outline the procedure for thawing frozen cells (BCL, CTL, PBMC).

II. SAFETY

This procedure needs to be carried out in the BSL2+ laboratory following all BL2+ regulations.

III. SCOPE

This procedure applies to all employees, students, contractors and visitors that work on thawing frozen cells.

IV. REQUIREMENTS

Training to be obtained by qualified trainer or Subject Matter Expertise (SME).

V. RESPONSIBILITIES

A. The Ragon Institute **qualified trainers** are responsible for the overall implementation of this procedure and ensuring compliance and for periodic review of this procedure. Updates if any may be initiated by the qualified trainers or Subject Matter Expertise (SME).
B. **All employees, students, contractors and visitors** are required to follow this procedure.

VI. PROCEDURE

Note: Cells are frozen in 10% DMSO in FCS.

To thaw them: keep everything cool.
Precool 15ml conical tubes, have FCS or R10 on ice.

1. Thaw cryopreserved tube with cells in water bath or in the palm of your hand until there is a small pellet of ice visible. **Don't** wait until the entire vial is thawed! Once you see the vial starting to thaw, wipe it with alcohol and transfer it into the hood along with a 15 ml conical tube and the FCS or R10. The vial will finish thawing in the time it takes you to do this. Ideally, you want the cells thawed for as little time as possible before they are spun down away from the DMSO. (DMSO is toxic to cells.)
2. Wipe the vial with alcohol, then transfer the vial into the hood. Transfer the cells into a precooled 15 ml conical tube.
3. Wash cryopreserves vial with FCS to get the remaining cells, then add 10 ml cold FCS.
4. Spin cells down for 10 minutes in cold (4□□C) centrifuge at 1500 RPM.
5. Aspirate off as much of the freezing solution as possible without taking a piece of the pellet.

6. Resuspend in 3 ml of R10 medium for counting. Take a small aliquot and place into a clean eppendorf tube.
7. Add 7 ml R10 medium (from this step it doesn't need to be cold) and spin again for 10 minutes at 1500RPM.
8. While cells are in the second spin, count the cells from the aliquot. See Protocol #59 Counting Cells.

BCL

After the second wash, aspirate off the media and add 5ml R20. Transfer into a T25 and place in the incubator.

CTLs

Have feeders solution ready (irradiated allogeneic PBMC in R10). After second wash, transfer cells to T25 upright with 20 million feeders /20 ml volume and anti-CD3 (12F6) at 0.1 μg/ml.
The next day, aspirate half of the volume and add approximately 6 ml R10/50. Then feed twice a week according to the regular schedule.
Remember to always make fresh medium and fresh made feeders for thawing CTLs.

PBMCs

For ELIspot we thaw in R10 (to decrease background).
NEW! - We have been using DNase to thaw PBMC with a great deal of success. After transferring the cells to a 15 ml conical (still in 1 ml volume), add 20 μl DNase (15000 Units stock) for about 15 seconds; add rest of thawing medium and spin down. This method increase yields significantly.

DNase is manufactured by Boehringer Mannheim (#107921)
To make stock solution, dilute in 3ml of sterile PBS.

Check incubator CO_2 and temperature levels once a week with the FYRITE kit.