Thawing and Quality Control Protocol for Human Peripheral Blood Mononuclear Cells (PBMCs), Bone Marrow Mononuclear Cells (BMMCs), & Ascites Cells

PURPOSE:

This document establishes a standard protocol for quality control testing of our cryopreserved cell samples.

THAWING AND QUALITY CONTROL PROCEDURE:

All steps involving pipetting are to be executed in a Class II biosafety cabinet. Cells are counted using a Nexcelom Bioscience Cellometer Vision for quality control purposes.

A. Preparation of media, reagents and equipment

1. Using a micropipette, aliquot 20µL of AOPI (acridine orange/propidium iodide) solution into one 0.5 mL black, light-protected microcentrifuge tube for each sample to be counted. Store at 4°C until ready to use. (AOPI Staining Solution catalog number CS2-0106-5mL, Nexcelom Bioscience)

2. For post-thaw QC, prepare Dulbecco's Modified Eagle Medium (DMEM) + 10% Fetal Bovine Serum (FBS) solution as follows:
    a. Mix the following:
        - 500mL DMEM (VWR catalog number 10-090-CV)
        - 50mL heat-inactivated FBS (VWR catalog number 45001-108)
        - 5.5mL pen/strep (pen: 10,000U/mL, strep: 10,000U/mL) (VWR catalog number 16777_164)
        - 5.5mL Fungizone (250µg/mL) (VWR catalog number 82026-728)
    b. Aliquot DMEM + 10% FBS into 1.8mL aliquots in 15mL conical tubes to be used to count post-thaw cells. Store tubes at 4°C until ready for use.

3. 37°C water bath.

4. Dry ice in a plastic storage cooler.

B. Viability and cell number measurement for post-thaw QC

1. Turn on water bath and set at 37°C.

2. Pull QC vials from liquid nitrogen storage and keep them on dry ice until ready for use.
    a. Samples must have been stored in the vapor phase of liquid nitrogen for a minimum of 24 hours before being pulled for QC purposes.
    b. QC vials will be 200µL aliquots.

3. Take out the number of 1.8mL aliquots of DMEM + 10% FBS needed to perform post-thaw QCs from the 4°C storage.
    a. Place tubes in a tube rack and warm to 37°C.
    b. Once warmed, remove from heat source and spray rack and tubes with 70% ethanol.
    c. Place tubes and rack in hood.

4. From the dry ice, remove a few of the QC vials and thaw the samples using the 37°C water bath. Work in small batches of no more than 4 or 5 aliquots at a time.
    a. Quickly move the sample vials through the water to speed thawing.
    b. Thaw until only a 2mm frozen crystal remains in the sample.

5. For each 200µL QC aliquot, do the following:
    a. Get one of the 1.8mL conical tubes and take ~300µL of DMEM + 10% FBS from the tube.
    b. Add the ~300µL DMEM + 10% FBS to the cryovial of containing 200µL of cells. Constantly stir while adding the media.
    c. Collect cells and media from the cryovial and slowly place back into the 15mL conical with constant stirring.
    d. Rinse the QC vial with ~300µL from the 15mL conical and put back into the 15mL conical to be sure to get all of the cells from the QC cryovial.

e. 200µL of cells + 1.8mL DMEM/FBS gives a 1:10 dilution of cells to media.

6. Add 20µL of thawed cell suspension to the AOPI solution to stain the cells. Mix well by gently pipetting up and down at least 3 times.
    a. The final concentration of acridine orange will be 0.5 µg/mL when mixed with the cells.
    b. The final concentration of propidium iodide will be 10 µg/mL when mixed with the cells.

7. Calculate the dilution factor.
    a. The dilution factor is 1:2 of cells to fluorescent stain.
    b. The dilution factor of the cell suspension is 1:10.
    c. By multiplying the two dilutions, the final dilution of the stained cells is 1:20.

8. Peel the plastic film off of both sides of a Cellometer Counting Chamber (Nexcelom Bioscience catalog number SD100).

9. Load 20µL into one port of the counting chamber, and insert the loaded slide into the instrument and count.