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Quality Control Protocol for Cryopreserved Cell Samples

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

QUALITY CONTROL PROCEDURE:

1) Add DMEM + 10% FBS (equilibrated to 37C) in a tube so that a final 1:10 dilution of sample and media is obtained. (Cell to Media Dilution Factor)
    a. Example: add 1.8 mL of media if the sample is 200 μl

2) After the sample has been stored in liquid nitrogen vapor phase for a minimum of 48 hours, remove one of the 200 μL test samples and place on dry ice.

3) Quickly thaw sample in a 37 - 40C water bath until a ~2 mm crystals remains. Quickly move sample through the water to speed thawing.

4) Spray the sample with 70% ethanol and dry using a ChemWipe.

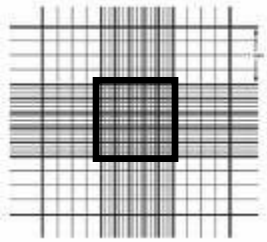
5) Slowly add ~300 μl of DMEM + 10% FBS from the prepared media in step #1 into the cryovial containing cells. Constantly stir while adding the media.

6) Collect cells and media from the cryovial and slowly place into the tube in step #1 with constant stirring.

7) Rinse the inside of the cryovial using media from the tube in step #1.

8) Place 10 μl of Trypan blue onto a small piece of parafilm. Mix 10 μl of well mixed cells with the Trypan blue on the parafilm. This creates a dilution factor of 2. (Trypan Blue Dilution Factor)

9) Load hemacytometer and count the number of viable cells under the microscope. Viable cells will likely be circular and have a blue outer layer with a clear inside. Nonviable cells will be blue on the outside and inside. Count the viable cells using the 10X objective in the area of the grid defined by the bold square below.



10) Perform the following steps to calculate the number of viable cells per mL:
    a. Cell Count x 10,000 = A
    b. A x 2 (Trypan Blue Dilution Factor) = B
    c. B x 10 (Cell to Media Dilution Factor) = Cells / mL

11) Record the viable cell count and the number of dead cells.

12) Clean the hemocytometer with 70% ethanol.

    a. Thoroughly disinfect the biosafety hood with 70% ethanol.