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Thawing Protocol for Cryopreserved Cell Samples

PURPOSE:
This document establishes a standard protocol for thawing our cryopreserved cell samples to ensure maximal viability post-thaw.

THAWING PROCEDURE:

1) Add 200-500 µl of media of interest or DNaseI (1 mg/mL) solution to a 15 or 50 mL conical for a 1 mL cryopreserved sample.
    a. DNaseI is resuspended in PBS (no Ca or Mg), filter (0.2 µm) sterilized, and aliquoted in single use vials.

2) Quickly thaw sample in a 37 - 40C water bath until a ~2 mm crystals remains. Move sample through the water to speed thawing.
    a. NOTE: samples are cryopreserved in media containing 10% DMSO.

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

4) Transfer cells from the cryovial to the conical containing the media of interest or DNaseI solution and mix (do not vortex). Rinse the inside of the cryovial with media of interest or DNaseI solution.
    a. Cell count and viability can be measured at this point by mixing 10 µl of cells with 10 µl of Trypan blue (or alternative dilution) and examining the cells on a hemocytometer.
        i. Recommended if the expected cell number is 1 million cells or less per mL.
    b. Quickly move to the next step since prolonged exposure to DMSO is toxic to cells.

5) Very slowly add 10-20 mL of PBS or media of interest that was previously equilibrated to 37C to the conical with constant stirring.
    a. If the sample is very clumpy, consider incubating the conical at 37C for 5-10 minutes to facilitate digestion of released DNA.

6) If the cell count and viability was not measured at step #4, then do so at this point.

7) Fill the conical with PBS or media to further dilute the DMSO.

8) Centrifuge the conical at 300xg for 5-10 minutes and remove the supernatant.

9) After this initial wash to remove the DMSO, cells can be used for various applications.

10) Suggestions:
    a. Add 0.1 mg/mL of DNaseI to the buffer or media if the cells are going to be separated through immunomagnetic isolation or during the first 16-24 hours after the cells are cultured.

b. Thoroughly wash away the DNaseI if hematopoietic cells are going to be placed in a methycellulose-based media.