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FREEZING CELLS FOR STORAGE IN LIQUID NITROGEN

1. Cells should be in log phase growth, for example, fed 1-2 days before. Generally, 2-10 ml of cells are used per freezing vial, and each vial contains 1 ml of cells in 10% DMSO (dimethyl sulfoxide).

2. Freezing stock is 20% DMSO 80% HoS. To prepare, add slowly while mixing 20 ml of DMSO to 80 ml of HoS (the order is important). Allow to age 1 day before use. It is stable for months. Store at 4°C.

3. Label freezing vials with cell line and date and enter in cell catalogue.

4. Spin cells (200 x g, 5 min, 10°) and aspirate supernatant. Add enough HoS to give 0.5 ml/frozen aliquot and place on ice. Add an equal volume of 20% DMSO 80% HoS as several additions, mixing after each one, over a period of 2 min. Keep on ice.

5. Aliquot 1 ml/vial and place vials on ice. As soon as all vials are filled, transfer to freezing apparatus and commence freezing. Arrange in the sequence in which they will be stored, to facilitate later rapid transfer to liquid nitrogen. The freezing apparatus is a 4'' thick sandwich of styrofoam which splits down the middle and holds 25 or more vials and is placed in a -80° freezer for 2 hr to overnight to allow a controlled rate of freezing. More apparatuses can be made with 1'' thick styrofoam pieces and a cork borer.

6. After freezing, quickly transfer vials into trays and place in liquid N_2. Make sure location is recorded.

7. Within one hour to one week after transfer to liquid N_2, test thaw one vial of each line and grow for several days. Log the fraction of live cells after 2 days. After cells have grown to maximum density, remove a supernatant sample and assay for antibody content and specificity. Store in freezer.

8. Thawing cells: It is essential to tick off each vial you thaw on the 'vials thawed' column of the frozen cell catalogue. If after thawing your cells, there are only 2 vials remaining, it is your responsibility to freeze more cells.

9. Thawing procedure: Rapidly thaw in 37° bath while vortexing until ice is just barely melted. Wipe vial with 70% EtOH. Transfer to a tube containing 1 ml growth medium on ice and while mixing add a further 9 ml of cold growth medium over 2 min. Centrifuge, aspirate, suspend in 2 ml medium, place in 24-well plate, and do 2 serial 5-fold dilutions of cells in 2 ml in the same plate.

Cells are usually plated initially in growth medium containing 20% serum, and in the next transfer are grown in the usual serum concentration.