

## MSC Cryopreservation

### Purpose:

Long-term storage of MSC preps. Depending on the investigation underway, and given donor-to-donor variability of these preparations, it may be advisable to bank an aliquot of the cells as a matter of course.

### Materials:

Tyrode's salt solution

Trypsin-EDTA

Incubator

Bovine calf serum (BCS)

Pipettes

Centrifuge tubes.

Benchtop centrifuge

FGF-supplemented complete medium.

Hemocytometer.

LN<sub>2</sub> compatible pen.

Freezing vials (Nunc)

Ice and ice bucket

Isopropanol-filled freezing apparatus (Thermo Fisher Scientific)

-80 °C freezer overnight.

Long-term LN<sub>2</sub> storage container and appropriate boxes, canes, etc.

### Method:

1. Aspirate spent medium from plates.
2. Rinse the cell layer with the appropriate volume (5 ml for a 56 cm<sup>2</sup> tissue culture dish) of Tyrode's salt solution to wash cells.
3. Repeat the rinse.

4. Add the appropriate volume of Trypsin-EDTA (4 ml for a 56 cm<sup>2</sup> tissue culture dish).
5. Return to the incubator for 5 – 7 minutes. Keep the time of exposure as brief as possible.
6. When the majority of the cells have become well rounded or have detached from the tissue culture surface, stop the reaction by adding bovine calf serum (BCS) equal to half the volume of Trypsin-EDTA.
7. Draw up the cell suspension with a pipette and, with the same pipette, use the suspension to gently wash the remaining cells from the dish. It is not necessary (nor desirable) to remove all of the cells from the dish, as most of the non-fibroblastoid cells (which are not likely to be MSCs) in these cultures are more trypsin-resistant than the spindle-shaped fibroblast-like cells. Thus, trypsinization represents, after attachment of fibroblastic cells to plastic, the second component of the process of selection of MSCs from the total marrow cell population.
8. Transfer the cell suspension from all of the cultures to an appropriate size centrifuge tube or tubes.
9. Spin in benchtop centrifuge at 500 x g for 5 min.
10. Resuspend pellet in 5 - 10 ml of FGF-supplemented complete medium.
11. Determine cell number with a hemacytometer.
12. Label a sufficient quantity of appropriately-sized freezing vials using a LN<sub>2</sub> compatible pen.
13. Spin cells in a benchtop centrifuge at 500 x g for 5 min.
14. Resuspend the cells at 10<sup>6</sup> cells per ml in freezing medium and place on ice immediately.
15. Aliquot cells to appropriate freezing vials (Nunc)
16. Cap vials and place in isopropanol-filled freezing apparatus (Thermo Fisher Scientific)
17. Transfer freezing apparatus to -80 °C freezer overnight.
18. Transfer frozen vials to long-term LN<sub>2</sub> storage container

### To thaw cryopreserved cells

1. Remove vial from LN<sub>2</sub> storage container
2. Thaw rapidly in a 37 °C water bath.
3. Transfer cell suspension to a 15 ml conical tube
4. Centrifuge for 5 minutes at 500 x g
5. Remove most of the freezing medium, resuspend pellet in 37 °C complete culture medium
6. Plate cells at 10<sup>6</sup> cells per T175 flask; do not handle the flask for at least 24 hours