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Neuron Freezing Medium

Neuron Freezing Medium contains DMSO and is optimized for the cryopreservation of neurons (human, rat and mouse).

Required Supplies

1. Neural Differentiation Medium (Catalog # 21004-250) – also used to maintain neurons
2. 0.05% Trypsin EDTA (Catalog # 41004-100)
3. 0.25% Soybean Trypsin Inhibitor (Catalog # 41005-100)
4. D-PBS with calcium and magnesium (Cat.# 41001-500)
5. Neuron Freezing medium for NSCs (Catalog # 21006-050)

Preparing NSCs for Cryopreservation

1. For all volumes refer to Table 1 below.
2. Working with multiple plates
    a. Only work with a stack of 4 x 100mm plates at a time
    b. Minimize time out of the incubator during the process
    c. Work swiftly but carefully to minimize cell loss
3. Remove the media from the plate and add trypsin
4. Take the plates of cells immediately to the microscope so that you can observe the trypsin action.

    NOTE: Trypsinization of neurons takes less than 2 minutes. Extended time in trypsin decreases viability.

5. Tap the dishes against the palm of your hand to dislodge the cells and when the cells are free floating, return the plates to the safety cabinet
6. Neutralize the trypsin with an equal volume of Soybean Trypsin Inhibitor, pipetting up and down to ensure single cell suspension, then transfer cell suspension into appropriate size conical tube.
7. Wash the plate with Neural Differentiation Medium (also used to maintain mature neurons) and add to the tube, pipetting up and down to ensure a homogenous cell suspension.
8. Take an aliquot for cell counting
    a. Take 50ul cell suspension and add it to 50ul Trypan Blue
    b. Pipette up and down
    c. Load10ul of cell suspension to both counting chambers of a hemacytometer
    d. Count the cells within the center grid
    e. Calculate the total cell count

    Cell count X 10,000 X 2 X Volume = Total cell count

    Note: 10,000 is a hemacytometer constant and 2 is the Dilution factor

9. Divide the total cell count by 4-5 x 10^6 to determine the number of cryovials needed for freezing down the neurons for long-term storage.
10. Pellet the cells in all the tubes by centrifugation at 2000 RPM for 5 minutes.

11. While the cells are in the centrifuge print the labels needed for each cryovial
12. Place one label on each cryovial.
13. After the centrifuge stops, resuspend the cell pellet in enough Neuron Freezing Medium to freeze 4-5 x 10^6 cells/ml as calculated in step 9 above.
14. Transfer 1ml Neuron Freezing cell suspension into each cryovial
15. Transfer the cryovials into a controlled rate freezer or a styrofoam container then place the container into a -80°C freezer over night
16. Next day transfer the vials of cells into the LN2 freezer for long-term storage

Table 1. Volumes of trypsin, Soybean trypsin inhibitor and media

Dish/Flask Size	Growth Area (cm^2)	Trypsin Volume	Soybean Trypsin Inhibitor Volume	Media Volume
100mm	58.1	2	2	2
60mm	21.3	1	2	1.5
35mm	9.6	0.5	1	1
6-Well	9.6	0.5	1	1
12-Well	3.8	0.3	1	1

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