

Neural Freezing Medium

Neural Freezing Medium contains DMSO and is optimized for the cryopreservation of neural stem cells (human, rat and mouse).

Required Supplies

1. Neural StemCell Growth medium (Catalog # 21001-250)
2. 0.05% Trypsin EDTA (Catalog # 41004-100)
3. 0.25% Soybean Trypsin Inhibitor (Catalog # 41005-100)
4. D-PBS without calcium and magnesium (Cat.# 41002-500)
5. D-PBS with calcium and magnesium (Cat.# 41001-500)
6. Neural Freezing medium for NSCs (Catalog # 21005-050)

Preparing hNSCs 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. PBS Wash: Remove old medium and add D-PBS w/o calcium and magnesium
4. Remove the PBS wash and add trypsin
5. Take the plates of cells immediately to the microscope so that you can observe the trypsin action.

NOTE: Trypsinization of hNSCs takes less than a minute. Extended time in trypsin decreases viability.

6. 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
7. Neutralize the trypsin with Soybean Trypsin Inhibitor, pipetting up and down to ensure single cell suspension, then transfer cell suspension into appropriate size conical tube.
8. Wash the plate with Neural StemCell Growth medium and add to the tube, pipetting up and down to ensure a homogenous cell suspension
9. Take an aliquot for cell counting
 - a. Take 50ul cell suspension and add it to 50ul Trypan Blue
 - b. Pipette up and down
 - c. Load 10ul of cell suspension to both counting chambers of a hemacytometer
 - d. Count the cells within the center grid
 - e. Calculate the total cell count

$$\text{Cell count} \times 10,000 \times 2 \times \text{Volume} = \text{Total cell count}$$

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

10. Divide the total cell count by 1.5×10^6 to determine the number of cryovials needed for freezing down the cells for long-term storage.
11. Pellet the cells in all the tubes by centrifugation at 2000 RPM for 5 minutes.

12. While the cells are in the centrifuge print the labels needed for each cryovial
13. Place one label on each cryovial.
14. After the centrifuge stops, resuspend the cell pellet in enough Neural Freezing Medium to freeze 1.5×10^6 /ml as calculated in step 10 above.
15. Transfer 1ml Neural Freezing cell suspension into each cryovial
16. Transfer the cryovials into a controlled rate freezer or a styrofoam container then place the container into a -80°C freezer over night
17. Next day transfer the vials of cells into the LN2 freezer for long-term storage

Table 1. Volumes of D-PBS , trypsin, Soybean trypsin inhibitor and media

Dish/Flask Size	Growth Area (cm ²)	D-PBS Wash Volume	Trypsin Volume	Soybean Trypsin Inhibitor Volume	Media Volume
100mm	58.1	8	1.5	2.5	2
60mm	21.3	5	1	2	1.5
35mm	9.6	2	0.5	1	1
6-Well	9.6	2	0.5	1	1
12-Well	3.8	1	0.3	1	1

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