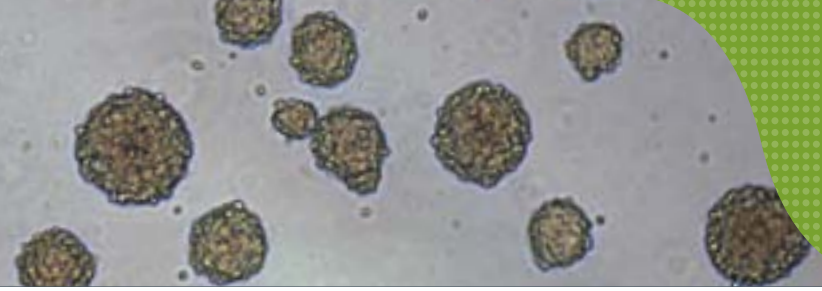

Summary Protocol

Cryopreservation of Neurospheres

Introduction

This protocol for the cryopreservation of neurospheres using controlled rate freezing containers is adapted from Gritti A, Galli R, and Vescovi AL. (2001) Cultures of Stem Cells of the Central Nervous System. In Federoff and Richardson A. (Eds) *Protocols for Neural Cell Culture*, 3rd Ed (pp 173-197) Humana Press Inc, Totowa, NJ.

Procedure for Cryopreservation

1) Preparation

a. Ensure that the freezing jar (5100 Cryo 1˚C Freezing Container, "Mr. Frosty"; Nalgene Catalog #5100-0001) is at room temperature (15 - 25˚C) and filled with isopropanol.

b. Prepare the freezing medium by adding DMSO to the culture medium to a final concentration of 10% (v/v). We recommend using the appropriate species-specific Complete NeuroCult™ Proliferation Medium (i.e. containing rh EGF, rh bFGF and Heparin as appropriate).

c. Label 2 mL cryogenic vials with name, date, passage number and cell type.

2) Cryopreservation

a. Collect neurospheres by gently pipetting.

b. Centrifuge at 110 x *g* for 10 minutes.

c. Remove supernatant and wash the cell pellet once with the appropriate species-specific Complete NeuroCult™ Proliferation Medium.

d. Centrifuge at 110 x *g* for 10 minutes.

e. Remove supernatant and resuspend the cell pellet quickly and gently in 1.5 mL freezing medium. Swirl gently to disperse the neurospheres.

f. Transfer the neurospheres into the pre-labelled cryovials.

g. Transfer cryovials into the freezing jar containing isopropanol.

h. Leave the freezing jar at -80˚C for a minimum of 4 hours, to allow a slow and reproducible decrease in temperature (-1˚C/minute).

i. Transfer cryovials into liquid nitrogen for long term storage.

3) Thawing

a. Warm the appropriate species-specific Complete NeuroCult™ Proliferation Medium in a water bath to 37˚C.

b. Quickly transfer cryovial(s) from liquid nitrogen to 37˚C water bath and leave until thawed. Swirl the vial to promote thawing.

c. Wipe the cryovial(s) thoroughly with 70% ethanol.

d. Slowly transfer the neurosphere cell suspension from the cryovial to a 15 mL polypropylene tube containing 5 mL warm Complete NeuroCult™ Proliferation Medium.

e. Centrifuge cell suspension at 110 x *g* for 8 minutes.

f. Remove most of the supernatant.

g. Gently resuspend cell pellet without dissociating the neurospheres in fresh warm Complete NeuroCult™ Proliferation Medium and place in a tissue culture flask of the appropriate size (depends on the quantity of cells thawed).

Notes for Successful Cryopreservation

- Do not let the neurospheres grow too large before harvesting for cryopreservation.
- Do not mechanically dissociate neurospheres before freezing. This will increase the number of dead cells, significantly lowering the viability of the thawed culture.

The same freeze-thaw protocol can also be applied to finely chopped embryonic CNS tissue. Please refer to Gritti A et al., (2001) for more information.

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