LINTERNA PC-12 CELL LINE

Culture Instruction Manual
(Considering T-75 Flasks)

Materials & Reagents Required

RPMI 1640 Medium, GlutaMAX™ Supplement (Ref: 61870010 from Gibco)
Heat-inactivated Horse Serum
Fetal Bovine Serum (FBS)
G418
DPBS (Ca^{2+} & Mg^{2+} free)
Incubator, 37 °C/5% CO_2.
Collagen-coated Tissue culture vessels (T-75 Flask)
Water Bath, 37 °C
15 mL tubes.
Centrifuge
Pipette
Ice

Complete Growth Medium

RPMI 1640 Medium, GlutaMAX™ Supplement
Heat-inactivated Horse serum (10%)
Foetal Bovine Serum (5%)
G418 (250 µg/ml)

The cell culture protocols described in this manual include the *in vitro* culture of. Any changes in the experimental conditions may have negative effects on cell survival and may yield abnormal cell experiment. For more information and for a complete list of Innoprot's reagents and products contact our customer service.

General Considerations: The protocols included in this manual are intended to serve as a guide only, and optimization of culture protocols is encouraged to ensure success.

Volumes used for this protocol are for a 75cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

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1.0 IMMEDIATELY UPON DELIVERY	
1.1	Remove vial from shipping container to check that it is still frozen.
1.2	Transfer frozen vial to liquid nitrogen until you are ready to thaw and begin cell culture.
2.0 THAWING CELLS – SEMIADHERENT CULTURE	
2.1	Prepare necessary "***Thawing medium***" and warm prior to plating cells: • RPMI 1640 Medium, GlutaMAX™ • 10% Heat-inactivated Horse Serum • 5% Fetal Bovine Serum
2.2	**Thaw cells rapidly**. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the oring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2.3	Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
2.4	Rinse the vial with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.
2.5	Transfer the vial contents to a 50 ml tube containing 9 mL complete growth medium. Centrifuge cells at 180 – 225 x g for 8-15 minutes at room temperature. Remove and discard supernatant. Resuspend cells in 5 mL complete growth medium. Break up cell clusters by gently aspirating cells through a 22g needle 4 or 5 times. (see the specific batch information for the culture recommended dilution ratio. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the collagen coated-culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
2.6	Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

3.0 MAINTENANCE OF THE CULTURE – SEMIADHERENT CULTURE	
3.1	Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium every 2-3 days.
3.2	Subculture when cell density reaches between 24 x 10^6 viable cells/ml or 90% confluent
4.0 SUBCULTURING – SEMI-ADHERENT CULTURE	
4.1	Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to room temperature. We do not recommend warming the reagents and medium at 37 ºC waterbath prior to use.
4.2	Rinse the cells with DPBS.
4.3	Add 8 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37ºC incubator for 1 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of FBS; transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); at the end of trypsinisation, with one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
4.4	Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 180 – 225 x g for 8-15 minutes at room temperature following 2.5 step again after centrifugation.
4.5	Count cells and plate cells in a new collagen-coated flask with cell density as recommended.