LINTERNA™ CELL LINES
GREEN FLUORESCENT NIH/3T3 CELL LINE

Culture Instruction Manual

Materials & Reagents Required

Dulbecco's Modified Eagle's Medium - high glucose (Ref: D6429 Sigma-Aldrich)
Fetal Bovine Serum (FBS)
Puromycin
DPBS (Ca^{2+} & Mg^{2+} free)
Trypsin/EDTA Solution
Incubator, 37 °C/5% CO_2.
Tissue culture vessels
Water Bath, 37 °C
15 mL tubes.
Centrifuge
Pipette
Ice

Complete Growth Medium

Dulbecco's Modified Eagle's Medium - high glucose (Ref: D6429 Sigma-Aldrich)
Fetal Bovine Serum (10%)
Puromycin (3 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.

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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	
2.1	Prepare necessary "***Thawing medium***" and warm prior to plating cells: • DMEM - high glucose (Ref: D6429 Sigma-Aldrich) • 10% FBS
2.2	Prepare coated flask (T-75 flask is recommended). Add 9 ml of DMEM and then add 1 ml of FBS (without selection antibiotic). Leave the flask in incubator minimum one hour at 37ºC incubator. .
2.3	**Thaw cells rapidly**. Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Do not allow sample to warm to 37ºC. Cryovials should be cool to the touch when removed from bath. Passive thaw is not recommended.
2.4	Remove the vial from the waterbath immediately, wipe it dry, and transfer it to a sterile field.
2.5	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.
2.6	Dispense the contents of the vial using 1 ml eppendorf pipette and gently resuspend the contents of the vial in T75 flask containing pre-warmed complete growth media.
2.7	Place the flask to the incubator
2.8	For best result, do not disturb the culture for 16 hours after the culture has been initiated. Change the growth medium (including the selection antibiotic) the next day to remove the DMSO and unattached cells, then every other day thereafter

3.0 MAINTENANCE OF THE 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 48 hours after establishing the subculture.
3.2	Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.
3.3	Subculture the cells when they are 80% confluent.
4.0 SUBCULTURING	
4.1	Warm medium, trypsin/EDTA 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	Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
4.4	Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: *To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.*
4.5	Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting
4.6	Add appropriate aliquots of the cell suspension to new culture vessels
4.7	Count cells and plate cells in a new flask at 37ºC with cell density as recommended: Subcultivation Ratio: Inoculate 3 to 5 X 10^3 cells/cm^2 Medium Renewal: Twice per week