LINTERNA CELL LINES

LINTERNA C2C12

Culture Instruction Manual

Required Materials & Reagents

- Dulbecco´s Modified Eagle´s Medium - High Glucose (D6429 Sigma-Aldrich)
- MEM Non-Essential Amino Acid Solution (100×) (M7145 Sigma-Aldrich)
- Fetal Bovine Serum (FBS) (F7524 Sigma-Aldrich)
- Antibiotic Antimycotic Solution (100×) (A5955 Sigma-Aldrich)
- Gentamycin (G1397 Sigma-Aldrich)
- Trypsin (25300-062 Gibco)
- G418 (A1720 Sigma-Aldrich)
- DPBS (Ca^{2+} & Mg^{2+} free) (D8537 Sigma-Aldrich)
- Incubator: 37 °C and 5% CO_2
- Cell culture vessels
- Water Bath, 37 °C
- 15 ml tubes
- Centrifuge (Beckman Coulter Allegra 6R centrifuge or similar)
- Pipette
- Ice

Complete Growth Medium

- DMEM
- MEM Non-Essential Amino Acid Solution 1X
- Antibiotic Antimycotic Solution 1X
- Gentamycin 1X
- Fetal Bovine Serum (10%)
- G418 (500 µg/ml)

General Considerations:

-The protocols in this manual are intended as a guide. Optimization of culture protocols is encouraged for better outcomes.

-Any changes to experimental conditions may negatively impact cell survival and produce abnormal results.

-For more information and for a complete list of Innoprot's reagents and products contact our customer service.

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1. IMMEDIATELY UPON DELIVERY

1.1. Remove the vial from the shipping container and check its integrity.

1.2. Store frozen cells in liquid nitrogen until use.

2. THAWING CELLS

2.1. Prepare the ***"Thawing medium"*** and warm it prior to plating cells:

- DMEM
- 10% FBS

2.2. **Thaw the cells rapidly**. Place the vial in a 37 °C water bath, hold and rotate the vial gently until the content is completely thawed. Do not allow sample to warm to 37 °C. The cryovials should be cool to the touch when removed from the bath. Passive thawing is not recommended.

2.3. Rinse the vial with 70% ethanol and wipe it to remove the excess.

2.4. Add 8 ml of pre-warmed media to a sterile 15 ml tube.

2.5. Transfer the cells to the 15 ml tube and mix gently.

2.6. Centrifuge at 1500 rpm for 5 minutes. Remove the supernatant and resuspend the cell pellet in "thawing medium" (DMEM+ 10% FBS).

2.7. Plate the cells in a T-25 flask and place the flask in the incubator.

2.8. For best results, do not disturb the culture for 24-48 hours.

3. MAINTENANCE OF THE CULTURE

3.1. Change the media to fresh medium supplemented with G418 (400-500 µg/ml) every 48-72h.

3.2. Subculture the cells when they are over 90% confluent.

4. SUBCULTURING

4.1. Warm medium, trypsin/EDTA solution and DPBS to room temperature. We do not recommend warming the trypsin/EDTA solution at 37 °C in the water bath prior to use.

4.2. Rinse the cells with DPBS.

4.3. Add 1 ml of trypsin/EDTA solution into the flask (T-25 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37 °C in the incubator for 2 minutes or until the cells are completely rounded up (check with an inverted microscope). Complete detachment may require gentle tap of the flask. Add 5 ml of complete growth medium to the flask and transfer the cells to a sterile 15ml tube. Add another 5 ml of complete growth medium to harvest the residual cells. Examine the flask under an inverted microscope to ensure the cell harvesting is successful. There should be less than a 5 % of attached cells.

4.4. Centrifuge at 1200 rpm for 5 min; discard the supernatant and re-suspend the cells in complete growth medium. Plate the cells in a new flask at the desired density.

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