VAMPIRO CELL LINES

VAMPIRO MDA-MB-231

Cell Culture Manual

Required Materials & Reagents

- RPMI 1640-Glutamax (61870-010ThermoFisher)
- 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)
- Puromycin (P9620 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

- RPMI 1640-Glutamax
- MEM Non-Essential Amino Acid Solution 1X
- Antibiotic Antimycotic Solution 1X
- Gentamycin 1X
- Fetal Bovine Serum (10%)
- Puromycin (10 µg/ml)

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Any changes in the experimental conditions may have negative effects on cells survival and may yield abnormal results. For more information and for a complete list of Innoprot's reagents and products contact our customer service.

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:
- RPMI 1640-Glutamax
- 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" (RPMI 1640-Glutamax + 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. MAINTENACE OF THE CULTURE

3.1. Change the media to fresh medium supplemented with Puromycin (10 µg/ml) every 48h.
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.

General Considerations: *The protocols included in this manual are intended to serve just as a guide. Optimization of culture protocols is encouraged to ensure success.*

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