Experiment time: 2025-03-05, 18:00 - 20:00

Cell Thawing

I. Objective of the experiment

To revive T24 cell lines and T24-RC48 cell lines with varying degrees of drug resistance, and transfer them into cell culture flasks.

II. Materials and reagents

2.1 Materials

Pipette tips and 15ml centrifuge tube were purchased from Axygen

Serological pipettes were purchased from a domestic company

Micropipettes: Eppendorf

T-25 cell culture flasks were purchased from Corning

Human bladder transitional cell carcinoma cell line (T24)

T24-RC48 cell lines with low, medium, and high drug resistance

2.2 Reagents

Dimethyl sulfoxide (DMSO) from WAK CHEMIE, Germany

McCoy's 5A medium from Shanghai Yuanpei Biotechnology Co., Ltd., China

Fetal bovine serum (FBS, Gibco)

III. Experimental instruments

Clean bench: Suzhou purification equipment factory

Benchtop low-speed centrifuge at room temperature: Eppendorf

HX-20 constant temperature metal bath (room temperature): Shanghai Huxi

IV. Experimental steps

4.1 The surface of the biosafety cabinet was wiped with alcohol wipes. All required reagents and consumables were placed inside the cabinet, which was then irradiated with UV light for 30 minutes for disinfection.

4.2 The frozen cell vial was rapidly thawed in a 37°C constant-temperature metal bath (pre-warmed) by gently agitating until just ice crystals remained (approximately 1 minute).

4.3 A 15 mL centrifuge tube was prepared with 5 mL of pre-warmed complete culture medium.

4.4 The cell suspension was aseptically transferred to the prepared centrifuge tube. Centrifugation was performed at 800 rpm for 5 minutes at room temperature.

4.5 After centrifugation, discard the supernatant and add fresh culture medium to resuspend the cells.

4.6 The resuspended cells were seeded into a new, sterile T-25 culture flask. Cell morphology and initial density were observed using an inverted microscope.

4.7 The culture flask was placed horizontally in a 37°C incubator with a 5% CO_2 humidified atmosphere.

4.8 Cell attachment and morphology were examined under an inverted phase-contrast microscope after 24 hours of incubation.

V. Photo of experimental operation:

Figure 1 Preparation of complete culture medium

VI. Experimental results

After 24 hours, both the T24 cell line and T24-RC48 cell line successfully resuscitated in the culture flask, exhibiting epithelial-like morphology with adherent growth. Notably, the T24 cell line and moderately and highly drug-resistant cell lines reached 100% confluence, while the low-drug-resistant cell line maintained approximately 40% confluence. Only a minimal number of floating (non-adherent) cells were observed in the medium.

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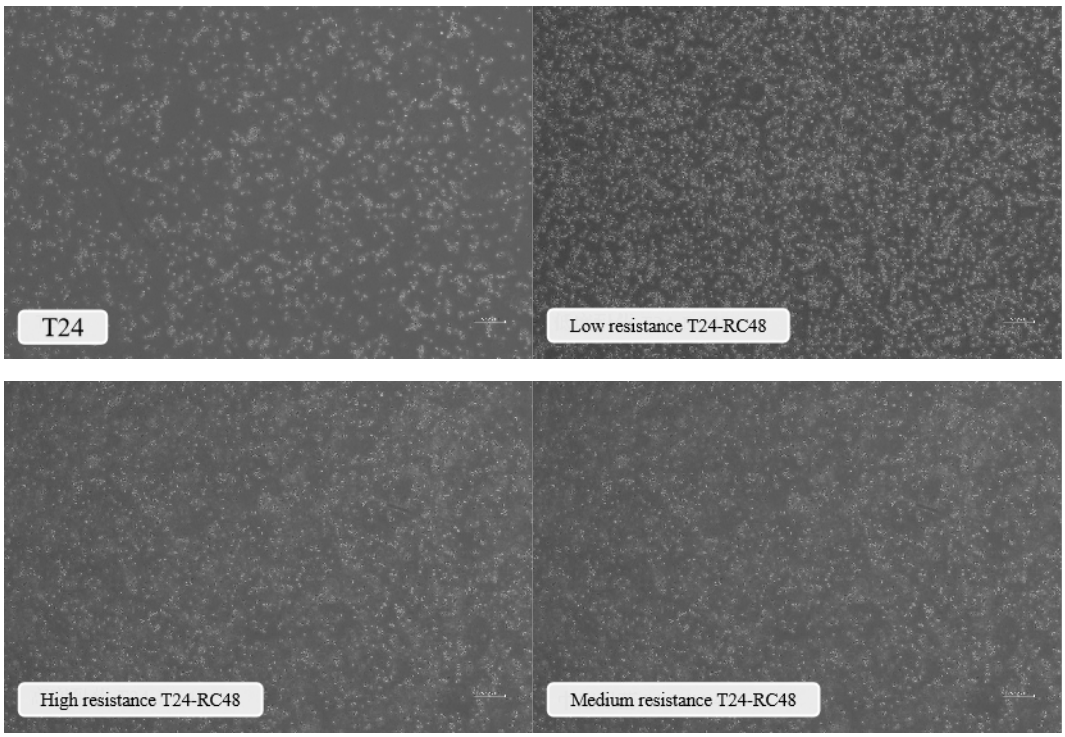

Figure 2. Morphology of cells immediately after thawing under an inverted phase-contrast microscope

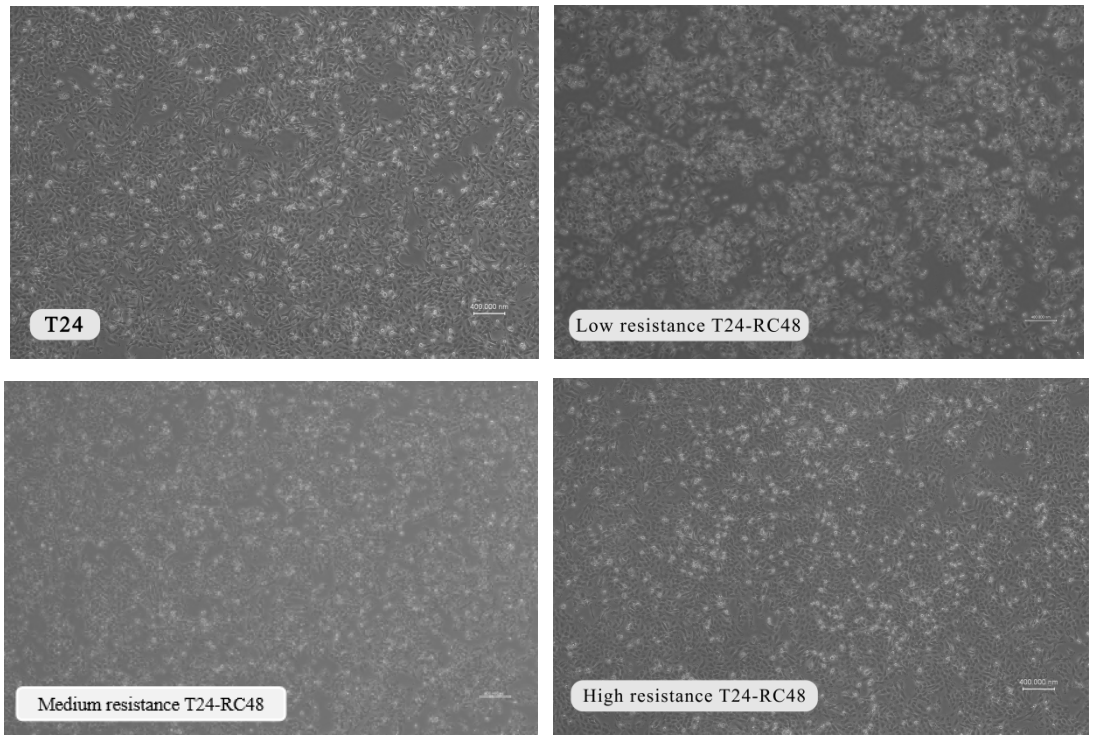

Figure 3. Morphology of thawed cells after 24 hours of culture under an inverted phase-contrast microscope

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VII. Results analysis

The high adherence rate and low number of non-adherent cells indicate that the thawing procedure was successful. The T24 parental cell line, as well as the moderately and highly drug-resistant T24-RC48 sublines, reached approximately 100% confluence, while the confluence in low-drug-resistant cell lines was approximately 40%. This discrepancy stems from variations in the quantity of cells cryopreserved during the freezing process.