

TM3 Cells | 305167

General information

Description TM3 Cells are a unique cell line derived from 11 to 13-day-old male mouse Leydig cells, exhibiting adherent growth properties. These cells are non-tumorigenic, as they do not cause tumors in immunosuppressed mice, although they can form colonies in semisolid medium. They express the gene for prostaglandin F2a and are characterized by several expression markers including Luteinizing hormone (LH), Epidermal Growth Factor (EGF), and positive markers for androgen, estrogen, and progesterone receptors. A notable feature of TM3 cells is their response to LH, which leads to an increase in cAMP production; however, they do not respond to follicle-stimulating hormone (FSH). The maintenance of LH responsiveness is serum lot-dependent. Additionally, in the presence of LH, these cells can metabolize cholesterol. They have been tested and found negative for ectromelia virus (mousepox), ensuring a high standard of safety for laboratory use.

Organism Mouse

Tissue Testis

Synonyms TM-3

Characteristics

Breed/Subspecies BALB/c

Age 11 to 13 days

Gender Male

Morphology Epithelial

Growth properties Adherent

Regulatory Data

Citation TM3 (Cytion catalog number 305167)

Biosafety level 1

NCBI_TaxID 10090

CellosaurusAccession CVCL_4326

Biomolecular Data

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Handling

Culture Medium DMEM:Ham's F12 (1:1), w: 3.1 g/L Glucose, w: 2.5 mM L-Glutamine, w: 15 mM HEPES, w: 0.5 mM Sodium pyruvate, w: 1.2 g/L NaHCO₃ (Cytion article number 820400a)

Supplements Supplement the medium with 2.5% FBS, 5% horse serum

Dissociation Reagent Accutase

Subculturing Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

Fluid renewal 2 to 3 times per week

Freeze medium As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at $300 \times g$ for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

Incubation Atmosphere

37°C , 5% CO_2 , humidified atmosphere.

Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately -78°C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about -150 to -196°C . Storage at -80°C is acceptable only as a short interim step before transfer to liquid nitrogen.

Quality Control & Molecular Analysis

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Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.