Product sheet

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
Disease	Normal testicular Leydig cells (non-tumorigenic; BALB/c mouse)
Metastatic site	Not applicable (normal, non-tumorigenic testicular cell line)
Applications	Leydig cell biology; testicular steroidogenesis; LH/cAMP signalling; androgen/estrogen/progesterone receptor studies; gonadotropin responsiveness; cholesterol metabolism; testicular development and function research
Synonyms	TM-3

Characteristics

Breed/Subspecies	BALB/c
Age	11 to 13 days
Gender	Male
Morphology	Epithelial
Cell type	Leydig cells
Growth properties	Adherent

Regulatory Data

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Citation	TM3 (Cytion catalog number 305167)
Biosafety level	1
NCBI_TaxID	10090
CellosaurusAccession	CVCL_4326
GMO Status	No genetic modification; wildtype mouse Leydig cell line derived from neonatal BALB/c testis by primary culture

Biomolecular Data

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
Doubling time	approx. 36 to 48 hours
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.
Split ratio	1 to 3
Seeding density	1 to 3 × 10^4 cells/cm^2
Fluid renewal	2 to 3 times per week
Post-Thaw Recovery	After thawing, plate the cells at 5 × 10^4 cells/cm^2 and allow at least 24–48 hours for adherence before the first medium change. Maintain serum lot-dependent LH responsiveness by validating each FBS lot for cAMP response to LH.

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.
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 x 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

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.