

**MCA-3D Cells | 400437**

**General information**

**Description**

The MCA-3D cell line is derived from primary mouse epidermal cultures that exhibit resistance to calcium-induced terminal differentiation. These cells were initially treated with the carcinogens N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) or 7,12-dimethylbenz[a]anthracene (DMBA), and subsequently exposed to 12-O-tetradecanoylphorbol-13-acetate (TPA). The resistance to terminal differentiation was assessed by elevating calcium levels in the culture medium to 1.2 mM, which selectively allows for the growth of transformed cells while normal cells typically undergo terminal differentiation and death.

The MCA-3D cell line displays an epithelial morphology and forms well-defined colonies in culture. Ultrastructural analysis reveals that MCA-3D cells contain keratin filaments and desmosomes, which are indicative of their epithelial origin and suggest maintenance of some degree of normal keratinocyte differentiation. However, the exact abundance of these structures can vary among subpopulations within the line.

MCA-3D cells have been tested for tumorigenicity by subcutaneous injection into syngeneic Balb/c neonates, with results indicating that this line is non-tumorigenic, even after prolonged culture in high calcium conditions. Additionally, the MCA-3D cells do not grow in soft agar, further supporting their non-malignant phenotype. Biochemical assays for gamma glutamyl transpeptidase (GGT) activity and transglutaminase activity have shown that MCA-3D cells are negative for GGT, and their transglutaminase activity does not correlate with tumorigenic potential, aligning with their non-tumorigenic classification.

Overall, the MCA-3D cell line serves as a model for studying the early stages of carcinogenesis and the factors that influence the progression from preneoplastic lesions to fully malignant tumors.

**Organism** Mouse

**Tissue** Skin

**Synonyms** MCA3D, MCa3D, MCA/3D, MCA 3D

**Characteristics**

**Breed/Subspecies** BALB/c

**Gender** Female

**Cell type** Keratinocyte

**Growth properties** Adherent

**Regulatory Data**

**MCA-3D Cells | 400437****Citation** MCA-3D (Cytion catalog number 400437)**Biosafety level** 1**NCBI\_TaxID** 10090**CellosaurusAccession** CVCL\_5797**Biomolecular Data****Handling****Culture Medium** Ham's F12, w: 1.0 mM stable Glutamine, w: 1.0 mM Sodium pyruvate, w: 1.1 g/L NaHCO<sub>3</sub> (Cytion article number 820600a)**Supplements** Supplement the medium with 10% FBS**Dissociation Reagent** TrypLE Express**Subculturing** Remove medium and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10ml for T75 cell culture flasks). Add TrypleExpress(1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at 37 degree Celsius for 15-20 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 5 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.**Seeding density** 0.5 to 1 x 10<sup>4</sup> cells/cm<sup>2</sup>**Fluid renewal** 2 to 3 times per week**Post-Thaw Recovery** After thawing, plate the cells at 5 x 10<sup>4</sup> cells/cm<sup>2</sup> and allow the cells to recover from the freezing process and to adhere for at least 24 hours.**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  $-150^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  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

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  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  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  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.