

CMK Cells | 305533

General information

Description

The CMK cell line is a human megakaryoblastic leukemia model established from a Down syndrome patient diagnosed with acute megakaryoblastic leukemia (AMKL). These cells are significant for research on megakaryocytic differentiation and leukemia associated with trisomy 21. CMK cells display markers typical of megakaryocytes, such as glycoproteins GPIIb/IIIa and GPIb, which are key components of platelet membranes. The presence of platelet peroxidase (PPO) activity, localized to the nuclear envelope and endoplasmic reticulum but not the Golgi apparatus, confirms the megakaryocytic lineage of these cells. Ultrastructural analysis reveals the existence of cytoplasmic granules resembling α -granules and demarcation membranes, further validating their megakaryocytic nature.

CMK cells are characterized by a complex karyotype, including near-tetraploidy and a specific translocation, der(17)t(11;17), also present in the patient's original leukemic cells. This genetic alteration links the cell line back to the disease state in the patient, providing a reliable model for studying AMKL pathogenesis. Additionally, the CMK cell line can respond to hematopoietic growth factors like interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF), which stimulate proliferation and differentiation into more mature megakaryocytic forms. Treatment with agents like phorbol ester (TPA) enhances the expression of megakaryocytic markers and induces morphological changes, including cytoplasmic segmentation resembling platelet formation. These features make CMK a valuable tool for investigating megakaryocytic lineage development, the effects of trisomy 21 on hematopoiesis, and the mechanisms of leukemogenesis in Down syndrome.

Organism

Human

Tissue

Peripheral blood

Disease

Myeloid leukemia associated with Down syndrome

Characteristics

Age

10 months

Gender

Male

Ethnicity

Japanese

Growth properties

Suspension

Regulatory Data

Citation

CMK (Cytion catalog number 305533)

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Biosafety level 1**NCBI_TaxID** 9606**CellosaurusAccession** CVCL_0216**Biomolecular Data****Mutational profile** Mutation: TP53, p.Asp49His (c.145G>C), heterozygous; Gene fusion: TP53-FXR2**Handling****Culture Medium** DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO₃, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)**Supplements** Supplement the medium with 20% FBS**Subculturing** Gather the suspension cells in a 15 ml tube and gently wash the adherent cells with PBS lacking calcium and magnesium (use 3-5 ml for T25 flasks and 5-10 ml for T75 flasks). Apply Accutase (1-2 ml for T25 flasks, 2.5 ml for T75 flasks) ensuring full coverage of the cell layer. Allow the cells to incubate at room temperature for 10 minutes. Following incubation, combine and centrifuge both the suspension and adherent cells. After centrifugation, carefully resuspend the cell pellet and transfer the cell suspension into new flasks containing fresh medium.**Seeding density** $3-5 \times 10^5/\text{ml}$ **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 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% CO₂, 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.