

imWilms1 Cells | 300412

Renseignements généraux

Description

The Wilms1 cell line was originally derived from a primary Wilms tumor, obtained from a patient diagnosed with large bilateral kidney tumors, a characteristic presentation of Wilms tumor (nephroblastoma). This cell line harbors a homozygous nonsense mutation in the WT1 gene (c.149 C>A, p.S50X), leading to the production of a truncated, non-functional WT1 protein. WT1 is a critical gene in kidney development, and its mutation is closely associated with the pathogenesis of Wilms tumor, particularly in tumors exhibiting stromal differentiation. Wilms1 cells display a stable karyotype without significant chromosomal abnormalities, and they are characterized by a mesenchymal phenotype, expressing vimentin while lacking epithelial markers like cytokeratin. The line shows a limited but significant capacity for mesenchymal differentiation, including the potential to differentiate into muscle-like cells under specific conditions, making it a crucial model for studying the molecular consequences of WT1 mutations.

To overcome the limited lifespan of the primary Wilms1 cells, the imWilms1 cell line was established by introducing a triple mutant SV40 large T antigen (U19dl89-97tsA58) into the original tumor cells, facilitating their immortalization. This modification allows imWilms1 cells to proliferate indefinitely while maintaining chromosomal stability, thereby offering a reliable model for long-term studies. The immortalized imWilms1 cells continue to exhibit the same WT1 mutation and retain the mesenchymal characteristics of the parent Wilms1 line.

In addition to its genetic and phenotypic features, the imWilms1 cell line has been extensively analyzed for its signaling pathway activity. Proteomic studies have revealed the phosphorylation and activation of several receptor tyrosine kinases (RTKs), including EGFR, PDGFR β , and AXL, with downstream activation of the MAPK signaling pathways. The consistent activation of these pathways in imWilms1 cells underscores their relevance for exploring targeted therapeutic strategies in Wilms tumor. Overall, imWilms1 serves as a robust and long-term model for investigating the molecular mechanisms underlying Wilms tumor development and progression, particularly those driven by WT1 mutations and aberrant signaling pathways.

Organism Human

Tissue Kidney

Disease Wilms Tumor

Synonyms IM-WT-1

Caractéristiques

Age 10 months

Gender Female

Ethnicity Caucasian

imWilms1 Cells | 300412**Morphology** Spindle-shaped**Cell type** Wilms cells**Growth properties** Adherent**Données réglementaires****Citation** imWilms1 (Cytion catalog number 300412)**Biosafety level** 1**NCBI_TaxID** 9606**CellosaurusAccession** CVCL_A5SN**GMO Status** GMO-S1: This imWilms1 human Wilms tumor line contains a triple-mutant SV40 T-antigen cassette enabling conditional immortalization for nephroblastoma research. This classification applies only within Germany and may differ elsewhere.**Données biomoléculaires****Mutational profile** WT1 mutation status: homozygous c. 149 C>A, p.S50x, LOH: 11p11-11pter, CTNNB1 mutation status: heterozygous TCT>TTT, p.S45F**Manipulation****Culture Medium** MSCGM kit (from Lonza)**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** 1 to 2 times per week

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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 $300 \times g$ 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.

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Contrôle de la qualité et analyse moléculaire

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.