Wilms8

300416

GENERAL INFORMATION

Description

The Wilms8 cell line was derived from a primary Wilms tumor in a pediatric patient with a germline WT1 mutation. This cell line is characterized by a homozygous nonsense mutation in the WT1 gene (c.1168 C>T, p.R390X), leading to a complete loss of WT1 function. WT1 is crucial for normal kidney development, and its inactivation is a common feature in certain aggressive subtypes of Wilms tumor, particularly those that exhibit mesenchymal differentiation. Wilms8, therefore, provides a valuable model for studying the effects of WT1 loss on tumorigenesis, especially in the context of Wilms tumors that arise with a pronounced stromal component.
In addition to the WT1 mutation, Wilms8 cells harbor a mutation in the CTNNB1 gene (p.S45A), which encodes β-Catenin, a key regulator of the Wnt signaling pathway. The mutation at serine 45 disrupts the normal phosphorylation process that leads to β-Catenin degradation, causing its stabilization and accumulation in the nucleus. This results in the constitutive activation of Wnt signaling, which drives cell proliferation and contributes to the oncogenic properties of the Wilms8 cell line. The interplay between WT1 loss and aberrant Wnt signaling in Wilms8 makes it a crucial model for understanding the molecular mechanisms underlying these pathways in Wilms tumor biology.
Wilms8 cells display a mesenchymal phenotype, characterized by the expression of vimentin and the absence of epithelial markers such as cytokeratin. This aligns with the stromal differentiation observed in the original tumor. The cells demonstrate a limited ability to undergo further mesenchymal differentiation, such as forming muscle-like cells under specific conditions. Proteomic analyses of Wilms8 have revealed the activation of multiple receptor tyrosine kinases (RTKs), including PDGFRβ and AXL, which are involved in key processes such as cell survival, migration, and proliferation. The activation of downstream signaling pathways, particularly the MAPK and PI3K/AKT pathways, further contributes to the aggressive characteristics of Wilms8 cells.
Overall, the Wilms8 cell line serves as an essential tool for investigating the molecular basis of Wilms tumor driven by WT1 loss and aberrant Wnt signaling. Its genetic and phenotypic features make it a robust platform for studying the interaction between these critical pathways and for identifying potential therapeutic targets in Wilms tumors with a stromal component.

Tissue	Kidney
Disease	Wilms tumor

Applications

In vitro cell culture model. Biochemical studies

CLASSIFICATION

Organism	Human
Sex	Male
Growth properties	Adherent
Biosafety level	1

CHARACTERISTICS

Morphology	Spindle-shaped
Cell type	Wilms cells

IDENTIFIERS

Citation

Wilms8 (Cytion catalog number 300416)

NCBI TaxID	9606
Cellosaurus	CVCL_A5SJ

EXPRESSION AND SUSCEPTIBILITY

Mutational profile

WT1 mutation status: homozygous c.1168C>T, p.390x, LOH: , CTNNB1 mutation status: heterozygous TCT>GCT, p.S45A

HANDLING AND CULTURE

Culture medium

MSCGM kit (from Lonza)

Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.
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.

Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
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.
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.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
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.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
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.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

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.

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.

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.

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.

DONOR

Donor age	8 months
Ethnicity	Caucasian

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Material Transfer Agreement required	Yes

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