

## Wilms2 Cells | 300413

## General information

## Description

The Wilms2 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.1084 C>T, p.R362X), which results in the production of a truncated, non-functional WT1 protein. The loss of functional WT1, a gene essential for kidney development, is a hallmark of certain subtypes of Wilms tumor, particularly those associated with mesenchymal or stromal differentiation. The Wilms2 cell line is a significant model for studying the tumorigenic processes driven by WT1 loss, especially in the context of Wilms tumors that retain other critical genetic features.

Wilms2 cells also carry mutations in the CTNNB1 gene, which encodes  $\beta$ -Catenin, a key component of the Wnt signaling pathway. These mutations, specifically affecting serine 45, lead to the stabilization and accumulation of  $\beta$ -Catenin, resulting in the constitutive activation of the Wnt pathway. This activation is a known driver of cell proliferation and tumorigenesis in Wilms tumor, making Wilms2 a valuable model for understanding how aberrant Wnt signaling contributes to the development and progression of tumors with WT1 mutations.

In terms of phenotype, Wilms2 cells exhibit a mesenchymal-like morphology, expressing vimentin and lacking epithelial markers such as cytokeratin. This aligns with the tumor's stromal characteristics and underscores the role of WT1 in regulating mesenchymal-epithelial transitions during kidney development. Proteomic analyses of Wilms2 have identified activation of several receptor tyrosine kinases (RTKs), including PDGFR $\beta$  and AXL, which are known to support tumor cell survival and proliferation. Additionally, downstream pathways such as MAPK and PI3K/AKT are also activated, further contributing to the malignant properties of Wilms2 cells.

Overall, the Wilms2 cell line serves as an essential tool for exploring the molecular mechanisms of Wilms tumor driven by WT1 loss and aberrant Wnt signaling. Its genetic and phenotypic characteristics provide a robust platform for investigating potential therapeutic targets and for understanding the role of key signaling pathways in the pathology of Wilms tumors with a mesenchymal component.

**Organism** Human

**Tissue** Kidney

**Disease** Wilms tumor

**Applications** In vitro cell culture model. Biochemical studies

## Characteristics

**Age** 1 year

**Gender** Male

**Ethnicity** Caucasian

**Morphology** Spindle-shaped

**Wilms2 Cells | 300413****Cell type** Wilms cells**Growth properties** Adherent**Regulatory Data****Citation** Wilms2 (Cytion catalog number 300413)**Biosafety level** 1**NCBI\_TaxID** 9606**CellosaurusAccession** CVCL\_A5SE**Biomolecular Data****Mutational profile** WT1 mutation status: homozygous c.149 C>A, p.R326x, LOH: 11p11-11pter, CTNNB1 mutation status: heterozygous del TCT>TAT, p.S45Y**Handling****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.**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.