

**Wilms6 Cells | 300415**

**Información general**

**Description**

The Wilms6 cell line was established from a primary Wilms tumor in a pediatric patient with a germline WT1 mutation. This cell line is defined by a homozygous nonsense mutation in the WT1 gene (c.1168 C>T, p.R390X), which results in a truncated and non-functional WT1 protein. WT1 is a critical regulator of kidney development, and its loss is strongly associated with Wilms tumor, particularly in cases displaying mesenchymal differentiation. The Wilms6 cell line is an important model for studying the tumorigenic effects of complete WT1 loss, particularly in the context of tumors that exhibit both epithelial and mesenchymal characteristics.

Wilms6 cells also carry a mutation in the CTNNB1 gene, specifically affecting serine 45 (p.S45F), a key site for phosphorylation that regulates  $\beta$ -Catenin degradation. This mutation leads to the stabilization and nuclear accumulation of  $\beta$ -Catenin, resulting in the constitutive activation of the Wnt signaling pathway. The aberrant activation of Wnt signaling is a known driver of cell proliferation and tumorigenesis in Wilms tumors, making Wilms6 a valuable tool for investigating the role of Wnt pathway dysregulation in tumors with WT1 mutations.

Phenotypically, Wilms6 cells display a mesenchymal morphology, with strong expression of vimentin and absence of epithelial markers such as cytokeratin, reflecting the stromal nature of the original tumor. These cells have been shown to possess limited but notable differentiation potential, including the ability to differentiate into muscle-like cells under specific conditions, which mirrors the mesenchymal differentiation observed in some Wilms tumors. Proteomic studies of Wilms6 have identified the activation of multiple receptor tyrosine kinases (RTKs), including PDGFR $\beta$  and AXL, which are involved in promoting cell survival, proliferation, and migration. The downstream activation of signaling pathways such as MAPK and PI3K/AKT further underscores the aggressive nature of this cell line.

Overall, the Wilms6 cell line serves as a crucial model for exploring the molecular mechanisms underlying Wilms tumor development, particularly in cases of complete WT1 loss combined with Wnt signaling activation. Its genetic and phenotypic characteristics make it an excellent platform for studying the interplay between WT1 deficiency and aberrant signaling pathways, providing insights into potential therapeutic targets for this aggressive tumor type.

**Organism** Human

**Tissue** Kidney

**Disease** Wilms tumor

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

**Características**

**Age** 15 months

**Gender** Male

**Ethnicity** Caucasian

**Wilms6 Cells | 300415****Morphology** Spindle-shaped**Cell type** Wilms cells**Growth properties** Adherent**Datos normativos****Citation** Wilms6 (Cytion catalog number 300415)**Biosafety level** 1**NCBI\_TaxID** 9606**CellosaurusAccession** CVCL\_A5SI**Datos biomoleculares****Mutational profile** WT1 mutation status: homozygous c.1168C>T, p.R390x, LOH: 11p11-11pter, CTNNB1 mutation status: homozygous del TCT, p.DS45**Manejo****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.

## Control de calidad y análisis molecular

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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.