

MDCK-SIAT1 Cells | 602281**General information****Description**

The MDCK-SIAT1 cell line is a modified version of the Madin-Darby Canine Kidney (MDCK) cells, engineered to express higher levels of human 2,6-sialyltransferase (SIAT1). This enzyme is responsible for the addition of sialic acid in an alpha-2,6 linkage to galactose on glycoproteins and glycolipids. The modification was performed to increase the expression of alpha-2,6-linked sialic acids, which are the primary receptors for human influenza viruses. This enhancement is critical as it makes the MDCK-SIAT1 cells more similar to the human airway epithelium, which naturally has a high concentration of these receptors. As a result, these cells offer a more physiologically relevant model for studying human influenza viruses and their interactions with potential antiviral compounds.

One of the significant applications of MDCK-SIAT1 cells is in the assessment of influenza virus sensitivity to neuraminidase inhibitors (NAIs), such as oseltamivir. Due to the increased presence of alpha-2,6-linked sialic acids, the MDCK-SIAT1 cells demonstrate improved sensitivity to NAIs compared to unmodified MDCK cells. This makes them an excellent tool for detecting resistance to these inhibitors, especially in low-passage-number clinical isolates of human influenza viruses. The MDCK-SIAT1 cell line allows for more accurate in vitro studies of drug efficacy and viral receptor interactions, providing valuable insights into the development of antiviral therapies and resistance mechanisms.

Organism Canine**Tissue** Kidney**Characteristics****Breed/Subspecies** Cocker Spaniel**Age** Adult**Gender** Female**Morphology** Epithelial**Growth properties** Adherent**Regulatory Data****Citation** MDCK-SIAT1 (Cytion catalog number 602281)**Biosafety level** 2

MDCK-SIAT1 Cells | 602281**NCBI_TaxID** 9615**CellosaurusAccession** CVCL_Z936**GMO Status** GMO-S1: This canine epithelial kidney cell line (MDCK-SIAT1) contains a pcDNA3.1GS construct encoding human 2,6-sialyltransferase (SIAT1), enabling expression of human-like sialylation patterns. The insert is stably present in MDCK cells. This classification applies only within Germany and may differ elsewhere.**Biomolecular Data****Protein expression** Transfected with ST6 beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1, SIAT1)**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 10% FBS and 1mg/ml G418**Dissociation Reagent** Accutase**Doubling time** 21 to 31 hours**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.**Seeding density** 2 to 4 x 10⁴ cells/cm²**Fluid renewal** 2 to 3 times per week**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.