

## CLS-439 Cells | 300150

**Description** Established from the primary bladder carcinoma of a 61-year-old male in 1998 by CLS.

**Organism** Human

**Tissue** Bladder

**Disease** Carcinoma

**Synonyms** CLS439

**Age** 61 years

**Gender** Male

**Ethnicity** Caucasian

**Morphology** Epithelial-like

**Growth properties** Adherent

**Citation** CLS-439 (Cytion catalog number 300150)

**Biosafety level** 1

**NCBI\_TaxID** 9606

**CellosaurusAccession** CVCL\_5982

**Tumorigenic** Yes, in nude mice

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<b>Culture Medium</b>	McCoy's 5a, w: 3.0 g/L Glucose, w: stable Glutamine, w: 2.0 mM Sodium pyruvate, w: 2.2 g/L NaHCO <sub>3</sub> (Cytion article number 820200a)
<b>Supplements</b>	Supplement the medium with 10% FBS
<b>Dissociation Reagent</b>	Accutase
<b>Doubling time</b>	35 hours
<b>Subculturing</b>	Remove medium and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10ml for T75 cell culture flasks). Add TrypleExpress (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at ambient temperature for 10 minutes. Carefully resuspend the cells, the addition of medium is optional but not necessary, and dispense into new flasks which contain fresh medium.
<b>Seeding density</b>	$1 \times 10^4$ cells/cm <sup>2</sup> will result in a confluent layer in about 3 days
<b>Fluid renewal</b>	2 to 3 times per week
<b>Post-Thaw Recovery</b>	The cells must rest for a minimum of 24 hours after thawing at 37 degree Celsius/5% CO <sub>2</sub>
<b>Freeze medium</b>	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.

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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.