

**Walker-256 (LLC-WRC 256) Cells | 500375****General information****Description**

The Walker-256 cell line is a rat carcinoma cell line that is widely used in cancer research, specifically in the study of tumor biology and chemotherapy. Originating from a mammary gland carcinoma of a rat, this cell line is particularly noted for its aggressive metastatic behavior, making it a valuable model for studying cancer progression and metastasis. It has been used extensively to investigate the mechanisms of tumor growth and the efficacy of anti-cancer drugs in vivo.

Walker-256 cells are adaptable to various environments, allowing them to be grown in a number of different animal models, which helps in the study of cancer biology in a systemic context. This cell line is instrumental in pharmacological studies, particularly those related to the development and testing of new chemotherapy agents. Researchers use Walker-256 to assess drug-induced cytotoxicity and to explore the potential mechanisms of action of novel therapeutic compounds. Its robust use in research provides critical insights into the dynamics of tumor growth and the systemic effects of tumors on host physiology.

**Organism**

Rat

**Tissue**

Mammary gland

**Disease**

Adenocarcinoma of the rat mammary gland

**Synonyms**

LLC-WRC 256, LLC-WRC256, Walker/LLC-WRC 256, Walker-Ca.256, Walker 256, W256, Lilly Laboratories Culture-Walker Rat Culture 256

**Characteristics****Breed/Subspecies**

Wistar

**Age**

Unspecified

**Gender**

Female

**Growth properties**

Suspension

**Regulatory Data****Citation**

Walker-256 (Cytion catalog number 500375)

**Biosafety level**

1

**NCBI\_TaxID**

10116

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**CellosaurusAccession** CVCL\_3537**Biomolecular Data****Handling**

|                       |                                                                                                                                                                                                                                                                                         |
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| <b>Culture Medium</b> | RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO <sub>3</sub> (Cytion article number 820700a)                                                                                                                                                                                    |
| <b>Supplements</b>    | Supplement the medium with 10% heat-inactivated FBS, 0.01 mg/mL Insulin, 4.5 g/L glucose, 1 mM Sodium pyruvate and 10 mM HEPES                                                                                                                                                          |
| <b>Subculturing</b>   | Maintain cultures by periodically adding or replacing the medium. Initiate cultures with a density of $5 \times 10^5$ cells/ml and keep the cell concentration within the range of $3 \times 10^5$ to $1 \times 10^6$ cells/ml for optimal growth.                                      |
| <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.

### 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.