



Walker-256 (LLC-WRC 256) | 500375

Culture  
Medium

RPMI 1640, w: 2.0 mM  $\beta$ -mercaptoethanol, w: 2.0 g/L NaHCO<sub>3</sub> (Cytion 820700a)

Supplements

10% FBS, 0.01  $\mu$ M dexamethasone, 4.5  $\mu$ M insulin, 1  $\mu$ M transferrin, 10  $\mu$ M HEPES

Subculturing

5-6  $\times 10^5$  cells per flask

Freeze  
medium

10% FBS + 10% DMSO

Thawing and  
Culturing Cells

1. Thaw cells rapidly in a water bath at 37°C.
2. Add cells to a flask containing 10 ml of culture medium.
3. Incubate cells for 24 hours at 37°C.
4. Remove medium and replace with fresh medium.
5. Seed cells into a 96-well plate at a density of  $1 \times 10^4$  cells per well.
6. Incubate cells for 24 hours at 37°C.
7. Harvest cells and analyze by flow cytometry.
8. Repeat steps 1-7 for each sample.

Incubation  
Atmosphere

37°C, 5% CO<sub>2</sub>

Flask Coating

None

Freezing  
Procedure

Freeze cells in a freezing medium at -80°C.

Shipping  
Conditions

Ship cells in a freezing medium at -80°C.

Product sheet

Walker-256 (LLC-WRC 256) | 500375

Storage  
Conditions

Store at 2-8°C. Do not freeze. Do not shake. Do not expose to light. Do not use after the expiration date. Do not use if the container is damaged. Do not use if the solution is cloudy or contains particles. Do not use if the solution is discolored. Do not use if the solution has a strong odor. Do not use if the solution is contaminated. Do not use if the solution is expired. Do not use if the solution is not at room temperature. Do not use if the solution is not in the original container. Do not use if the solution is not sealed. Do not use if the solution is not in the original container. Do not use if the solution is not sealed. Do not use if the solution is not in the original container. Do not use if the solution is not sealed.

Do not use if the solution is cloudy or contains particles. Do not use if the solution is discolored. Do not use if the solution has a strong odor. Do not use if the solution is contaminated. Do not use if the solution is expired. Do not use if the solution is not at room temperature. Do not use if the solution is not in the original container. Do not use if the solution is not sealed. Do not use if the solution is not in the original container. Do not use if the solution is not sealed.

Sterility

The product is sterile. Do not use if the solution is cloudy or contains particles. Do not use if the solution is discolored. Do not use if the solution has a strong odor. Do not use if the solution is contaminated. Do not use if the solution is expired. Do not use if the solution is not at room temperature. Do not use if the solution is not in the original container. Do not use if the solution is not sealed. Do not use if the solution is not in the original container. Do not use if the solution is not sealed.

Do not use if the solution is cloudy or contains particles. Do not use if the solution is discolored. Do not use if the solution has a strong odor. Do not use if the solution is contaminated. Do not use if the solution is expired. Do not use if the solution is not at room temperature. Do not use if the solution is not in the original container. Do not use if the solution is not sealed. Do not use if the solution is not in the original container. Do not use if the solution is not sealed.