



MRC-5 Cells

Catalog #: MRC001

Cell #: >5x10⁵ cells

Storage: Liquid Nitrogen until ready for culture.
While culturing keep in 37°C CO₂ incubator (95% air, 5% CO₂)

Product Format: Frozen Vial

GENERAL INFORMATION

MRC-5 cells are human fetal lung fibroblast cells. MRC-5 cells were derived from embryonic lung tissue of 14-week-old Caucasian male fetus in 1966. These diploid cells have been used extensively in vaccine production and other research. It is recommended to culture these cells following the protocols described below with the potential to reach up to 45 doubling populations.

Product is for Research use only.

Frozen Vials are shipped in a Dry Ice Package.

STATEMENT

Handling human tissue derived products is potentially bio-hazardous, despite testing negative for HIV, HBV, and HCV DNA. Nonetheless, proper precautions must be taken to avoid inadvertent exposure.

SPECIAL NOTES:

- We strongly advise our customers to use medium and related products recommended by Neuromics, because our cells were grown and adapted using our products.
- The growth featured of our cells cannot be guaranteed if the specific growth mediums stated in our datasheets are not used.
- Due to the sensitive nature of primary cells and cell lines, all quality related issues about the cells must be reported back to us within one month after receiving the products. Cells will not be replaced after the one-month period.

UNPACKING AND STORAGE INSTRUCTIONS

1. Check all containers for leakage or breakage. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.
2. Complete medium: The base medium for this cell line is Lung Fibroblast Cell Media (CCM005). To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

HANDLING PROCEDURE

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability. Thaw the vial by gentle agitation in a 37°C water bath. To

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reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

1. Remove the vial from the water bath as soon as the contents are thawed and decontaminate by dipping in or spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
2. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium (see the specific batch information for the culture recommended dilution ratio) and spin at approximately 125 x g for 5 to 7 minutes.
3. Resuspend cell pellet with the recommended complete medium. and dispense into a 25 cm² or a 75 cm² culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells.
4. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

PROTOCOL FOR THAWING THE CELLS AND SUBCULTURE

Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

Note: Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium. Briefly rinse the cell layer with Ca⁺⁺/Mg⁺⁺ free Dulbecco's phosphate buffered saline (D-PBS) solution to remove all traces of serum which contains inhibitor.
2. Add 2.0 to 3.0 mL of Trypsin-EDTA solution (CCM006) to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
 - a. **Note:** To avoid clumping, do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.
3. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of cell suspension to new culture vessels.
6. Incubate cultures at 37°C.
 - a. **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended.
 - b. **Medium Renewal:** Twice per week

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