PRODUCT DATA SHEET

PRODUCT: glioblastoma multiforme cell line with introduced EGFRvIII (point mutation C311Y) (T96)

CATALOG NUMBER: CL 01007-CLTH

SHIPPED IN: dry ice

STORAGE: Liquid nitrogen

QUANTITY & CONCENTRATION:

1 mL, 1 x 10^6 cells/mL in DMEM with 10% FBS and 10% DMSO

PHYSICAL FORM:

T96 EGFRviii cell lines are provided to customers in vials containing >1.0e7 cells/mL

BACKGROUND/DESCRIPTION:

The T96 cell line is established from human glioblastoma multiforme. The T96/EGFRvIII cell line stably expresses mutated EGFRvIII with additional point mutation C311Y in the truncated EGFR variantand neomicin-resistance gene. Both, mutated EGFRvIII and neomicin-resistance genes are under CMV promoter control, allowing for high levels of protein expression.

QUALITY CONTROL

This cryovial contains at least 1.0 × 107 T96/EGFRvIII cells as determined by morphology and viable cell count. The T96/EGFRvIII cells are tested free of microbial contamination.

MEDIUM

Complete Growth Medium: the base medium for this cell line is: DMEM (high glucose) with L-glutamine.
To make the complete growth medium, add the following components to the base medium: 10% fetal bovine serum (FBS) and 1% Penicillin/streptomycin.

UNPACKING & STORAGE INSTRUCTIONS

1. Check all containers for leakage or breakage.
2. Thaw the frozen cryovial according to subculturing procedure.

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3. Optimally: Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below 80°C, preferably in liquid nitrogen vapor, until ready for use.

HANDLING PROCEDURE FOR FROZEN CELLS

Establishing the T96/EGFRvIII cell cultures:

1. Place 10 mL of medium (as above) in a 15-mL conical tube.
2. Thaw the frozen cryovial in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol.
3. Transfer the cells to the conical tube containing the medium.
4. Centrifugation at 1100 rpm for 7 minutes at room temperature and then remove the medium.
5. Resuspend the cells in the fresh medium and transfer to a T-75 tissue culture flask.
6. Place the cells in a 37°C incubator at 5% CO2. Monitor the cell density daily.

SUBCULTURING PROCEDURE

1. Discard culture medium.
2. Briefly rinse the cell layer with PBS and discard it.
3. Add 1 mL 0.05% (w/v) Trypsin - 0.53 mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 minutes). To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. 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. Centrifuge cells 200xg for 5 min and suspend cells in fresh Complete Growth Medium.
6. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended.
Medium Renewal: Thirce per week.

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SAFETY PRECAUTION

AMS Biotechnology Europe recommends using protective gloves and clothing and wearing a full face mask always when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. During thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.
The product should be handled by trained personnel observing good laboratory practices. It is important to avoid breathing vapor, avoid skin contact or swallowing.

BIOSAFETY LEVEL: 1

Appropriate safety procedures should always be used with this material. Please check all safety procedures required in your country.

WASTE DISPOSAL

It is highly recommended that waste always be returned to special company responsible for utilizing such type of waste.

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