PRODUCT DATA SHEET

PRODUCT: **CLTH EGFR/EGFRvIII in selection**

CATALOG NUMBER: **CL 01011-CLTH**

SHIPPED IN: dry ice

STORAGE: Liquid nitrogen

Note: For best results begin culture of cells immediately upon receipt. If this is not possible, store at -80ºC until first culture. Store subsequent cultured cells long term in liquid nitrogen.

UANTITY & CONCENTRATION: 1 mL, 1 x 10^7 cells/mL in DMEM with 10% FBS and 10% DMSO

ORIGIN: Human

Original line from human embryonic kidney

QUANTITY & CONCENTRATION:

1 mL, 1 x 10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO

PHYSICAL FORM

CLTH EGFR WT EGFRvIII cell lines are provided to customers in vials containing > 1 x 10^6 cells/mL

BACKGROUND/DESCRIPTION

The CLTH EGFR WT/ EGFRvIII were stably transfected with genomic integration of a plasmid coding for EGFRvIII receptor protein and are expressing both Wild Type (WT) and mutated (vIII) form of Epidermal Growth Factor Receptor (EGFR). The expression of EGFR was confirmed by Western Blotting. The EGFR WT EGFRvIII cells are resistant to antibiotics Puromycin and G418 used as selection markers.

QUALITY CONTROL

This cryovial contains at least 1.0 × 10^6 CLTH EGFR WT EGFRvIII cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The CLTH EGFR WT EGFRvIII cells are tested free of microbial contamination.

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MEDIUM

Complete Growth Medium: the base medium for this cell line is: DMEM (high glucose) with L-glutamine (MED 02001-CLTH)

To make the complete growth medium, add the following components to the base medium: 0.1 mM MEM NonEssential Amino Acids (NEAA), 1% Pen-Strep, (optional),20 µg/mL Gentamicin (optional), 2 µg/mL Puromycin , 200 µg/mL G418.

Freeze Medium: 70% DMEM, 20% FBS, 10% DMSO.

UNPACKING & STORAGE INSTRUCTIONS

1. Check all containers for leakage or breakage.
2. Thaw the frozen cryovial according to subculturing procedure.
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 CLTH EGFR WT EGFRvIII Cultures from Frozen Stock

1. Place 10 mL of complete DMEM growth medium in a 50-mL conical tube. Thaw the frozen cryovial of cells within 1–2 minutes by gentle agitation in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol.
2. Transfer the thawed cell suspension to the conical tube containing 10 ml of growth medium.
3. Collect the cells by centrifugation at 1000 rpm for 5 minutes at room temperature. Remove the growth medium by aspiration.
4. Resuspend the cells in the conical tube in 15 mL of fresh growth medium by gently pipetting up and down.
5. Transfer the 15 mL of cell suspension to a T-75 tissue culture flask. Place the cells in a 37°C
1. incubator at 5% CO2.
6. Monitor cell density daily. Cells should be passaged when the culture reaches 95% confluence.

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, 5% CO_2

SAFETY PRECAUTION

Celther Polska Sp. z o.o. highly 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

Celther Polska higly recommends that waste always be returned to special company responsible for utilizing such type of waste.

CELTHER POLSKA SP. Z O.O. WARRANTY

The viability of Celther Polska products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. Celther Polska outlines the list of media formulation that has been found to be effective for this strain. While other, unspecified media may also give satisfactory results, a change in media or the absence of an additive from the Celther recommended media may cause problems with recovery, growth and/or function of this strain. If an alternative medium formulation is used, the Celther warranty for viability is no longer valid.

DISCLAIMERS / LEGAL INFORMATION

This product is intended for laboratory research purposes only. It is not intended for use in humans.
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CONTACT INFORMATION

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