PRODUCT DATA SHEET

PRODUCT: **DKMG/EGFRvIII**

CATALOG NUMBER: **CL 01008-CLTH**

SHIPPED IN: dry ice

STORAGE: Liquid nitrogen

QUANTITY & CONCENTRATION:

1 mL, 1 x 10^6 cells/mL in DMSO

ORIGIN: Human

PHYSICAL FORM

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

BACKGROUND/DESCRIPTION

The DKMG cell line is a permanent line established from cells derived from the glioblastoma. Cells were stably transduced with a viral vector coding for EGFRvIII receptor protein that underwent genomic integration. Cells express mutated (vIII) form of Epidermal Growth Factor Receptor (EGFR). DKMG cells show mutation and amplification of EGFR at genomic level, which is responsible for the expression of EGFRvIII mRNA and protein, in contrast to the majority of glioblastoma-derived cell lines where expression of mutatant EGFR gene amplicons is silenced. The performed biotechnological modification results in the EGFRvIII overexpression in all cells of the DKMG/EGFRvIII cell line.

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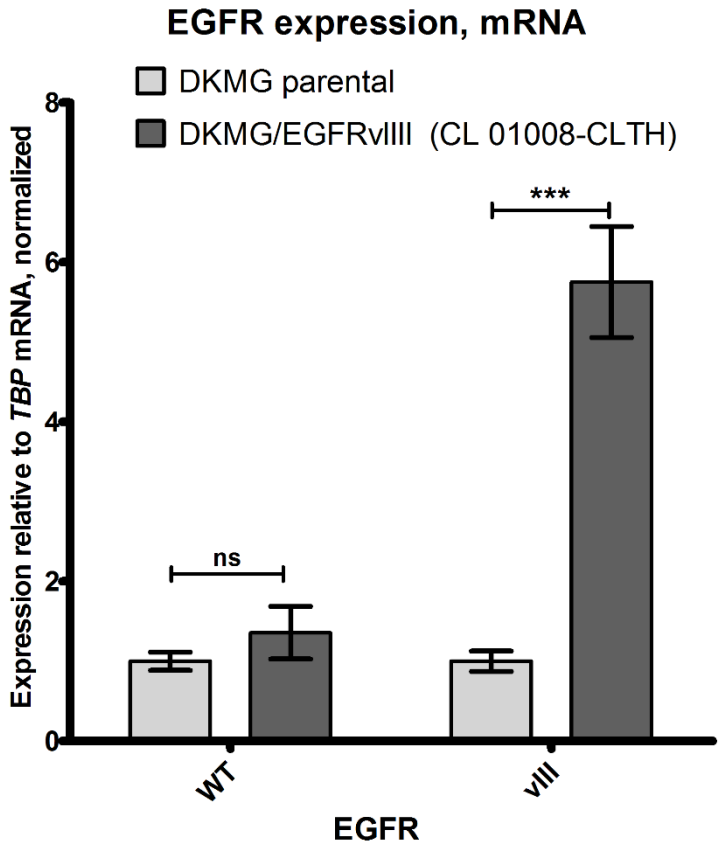
qPCR analysis of the DKMG parental and DKMG/EGFRvIII (CL 01008-CLTH) cell lines

cDNA obtained by reverse transcription of the mRNA obtained from DKMG/EGFRvIII (CL 01008-CLTH) and DKMG parental cell lines was used to assess wild-type EGFR and EGFRvIII transcript levels. Results are normalized to expression levels observed in the DKMG parental line. Pool of cDNA originating from numerous cancer tissues positive for EGFRvIII was used as a reference standard.



One-tailed Student's t-test was used for statistical analysis; ***, $p<0.005$; ns, not significant.

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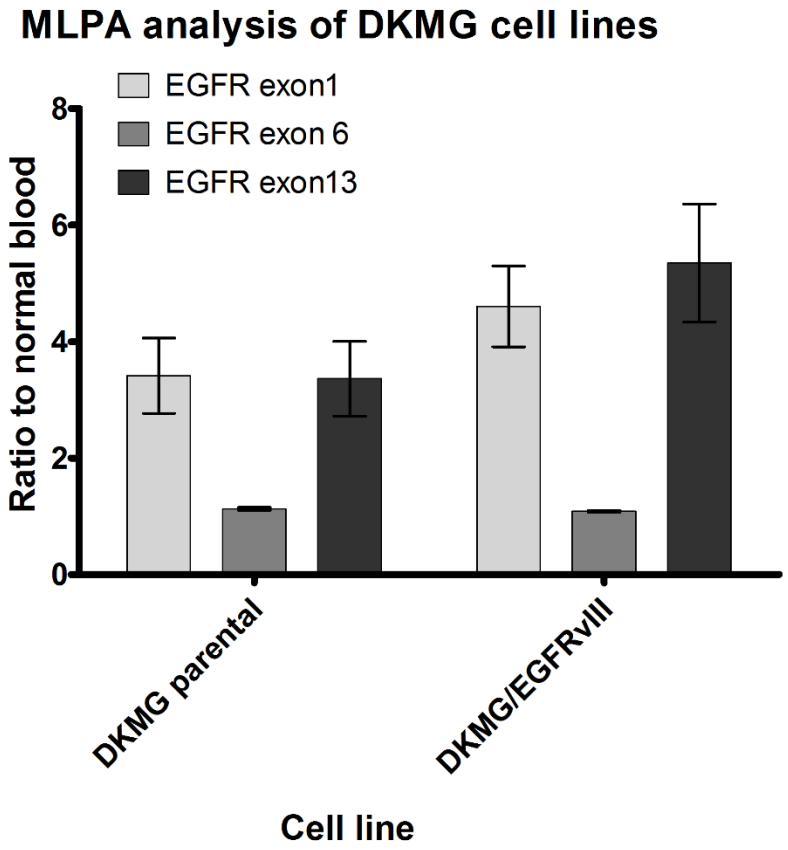
MLPA analysis of the DKMG parental and DKMG/EGFRvIII (CL 01008-CLTH) cell lines

Probes against exons 1 and 6 are elevated in both cell lines, in contrast to exon 6 - characteristic for exon 2-7 deletion in EGFRvIII. DKMG parental has EGFRvIII amplicons as described in the literature (Del Vecchio et al., 2012), whilst introduction of exogenous EGFRvIII increases it further



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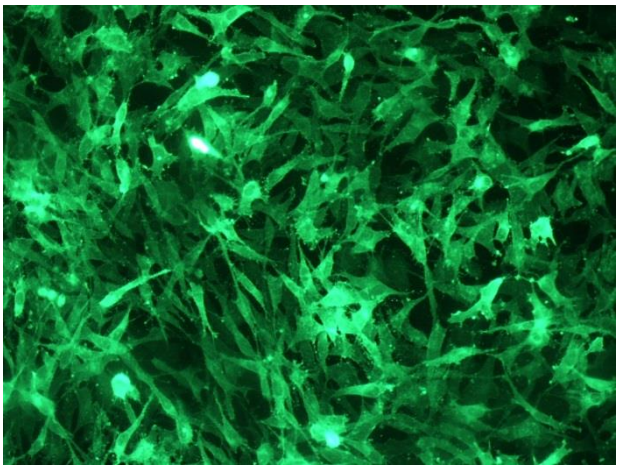
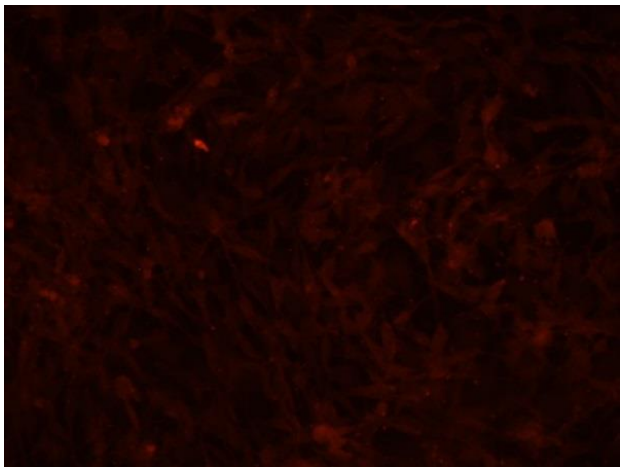
EGFRvIII and EGFRwt expression in DKMG/EGFRvIII cell line (CL 01008-CLTH)



(A) EGFRwt + EGFRvIII. DK-MG/EGFRvIII cell line



(B) EGFRwt. DK-MG/EGFRvIII cell line

Figures compare signal emanating from antibodies staining total EGFR (EGFRwt + EGFRvIII) (A) and EGFRwt (B), with the same exposture times in both cases. The difference in intensities is attributed to EGFRvIII expression, and confirmed by western blot analysis.

In the cell line DKMG/EGFRvIII, EGFRvIII expression is at least as high as that of EGFRwt.

QUALITY CONTROL

This cryovial contains at least 1.0×10^6 DKMG/EGFRvIII cells as determined by morphology and viable cell count. The DKMG/EGFRvIII cells were tested free of microbial contamination prior to shipment.

MEDIUM

Complete Growth Medium: the base medium for this cell line is: RPMI 1640. 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.
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.

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HANDLING PROCEDURE FOR FROZEN CELLS

Establishing the DKMG/EGFRvIII cell culture:

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. Centrifuge 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, 5% CO_2

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

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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