GeneCopoeia™
Expressway to Discovery

Datasheet for HCT116/EGFR ΔE746-A750 cancer marker mutation cell line (heterozygote)

Catalog number: SL702

Product: HCT116/EGFR ΔE746-A750 cancer marker mutation cell line (heterozygote)

Description: This product is a HCT116 cell line genetically modified using CRISPR to have one allele with a 15 bp deletion in exon 19 of EGFR and a puromycin marker in intron 18 of EGFR for selection, and the other allele with a wild type EGFR.

Genotype: EGFR (ΔE746-A750, Puro/+)

Genomic Mutation: GGAATTAAGAGAAGCAA -> GA

Quantity: 1 vial of 2 x 10^6 cells; frozen

Shipping conditions: Dry ice

Storage conditions: Liquid nitrogen vapor phase. Remove the item from the dry ice packaging and check all items for damage and leakage. Place immediately into storage at or below -140 ^{0}C, preferably into the liquid nitrogen vapor phase, until use.

Source of parental line:

HCT116
Organism: *Homo sapiens*, human
Tissue: colon
Cell Type: epithelial

Quality control: >95% viability before freezing. All cells were tested and found to be free of mycoplasma, bacteria, viruses, and other toxins.

Safety instructions: To ensure safety, protective gloves, clothing, and a face mask should be worn when handling frozen vials. Some leakage may occur into the vial during storage. The liquid nitrogen will be converted to gas upon thawing. Due to the nature of nitrogen gas, pressure may build within the vial and possibly result in the vial exploding or losing its cap. This may cause flying debris.

Thawing procedure: The vial of cells should be thawed in a 37 ºC water bath with gentle agitation. For optimal performance, the vial should be thawed in under two minutes. Ensure that the cap of the vial did not loosen upon thawing, and re-tighten as needed. Spray the vial with 70% EtOH and wipe off. Repeat. Using aseptic technique, add the contents of the vial to 9 ml of complete growth medium

(without selection). Centrifuge for 5 min. at 250 xg. Aspirate the medium, being careful not to disturb the pellet. Resuspend in 10 mL of complete growth medium, and place into a culture vessel of your choice. Only add selection to the medium after 24 hours in culture.

Culture conditions:

Complete Growth Medium

The base medium for this cell line is RPMI1640. For optimal growth and maintenance of selection, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Selection

Puromycin to a final concentration of 0.6 µg/mL.

Culture temperature:

37 ºC with 5% CO_2.

Subculture

Replace culture medium with selection-free medium and incubate for up to 6 hours. Rinse the cells and split at 1 x 10^5 viable cells/mL to 1 x 10^6 cells/mL.

Cryopreservation

Freeze slowly in complete growth medium supplemented with 5% (v/v) DMSO.

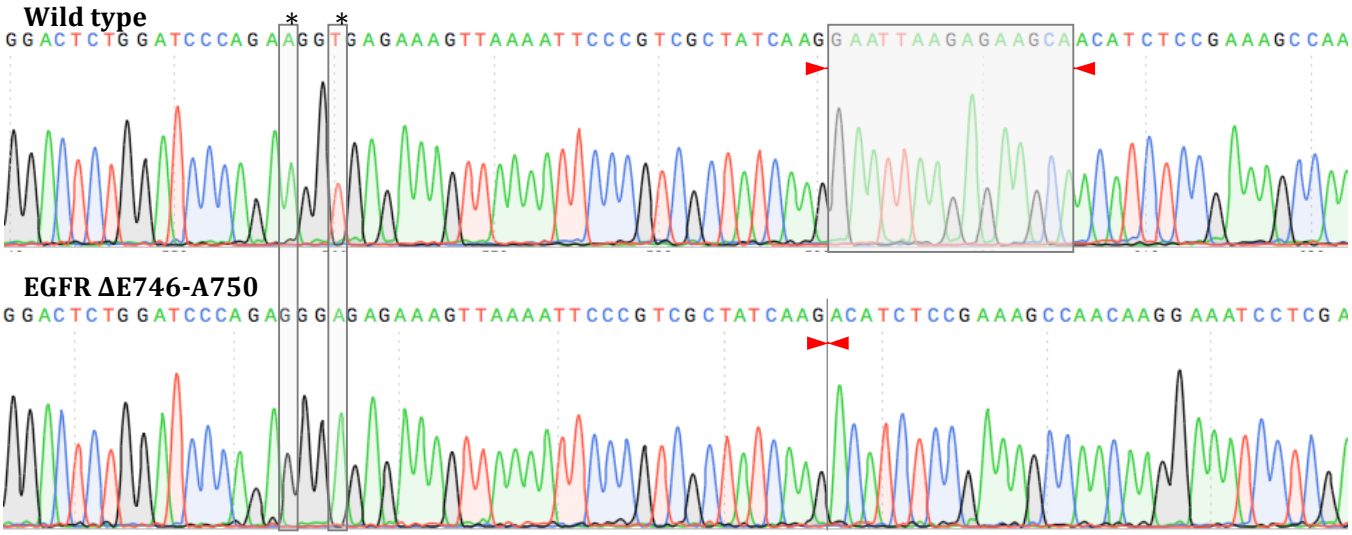
Mycoplasma

Negative (MycoAlert Mycoplasma Detection Kit from Lonza).

Product Quality Control

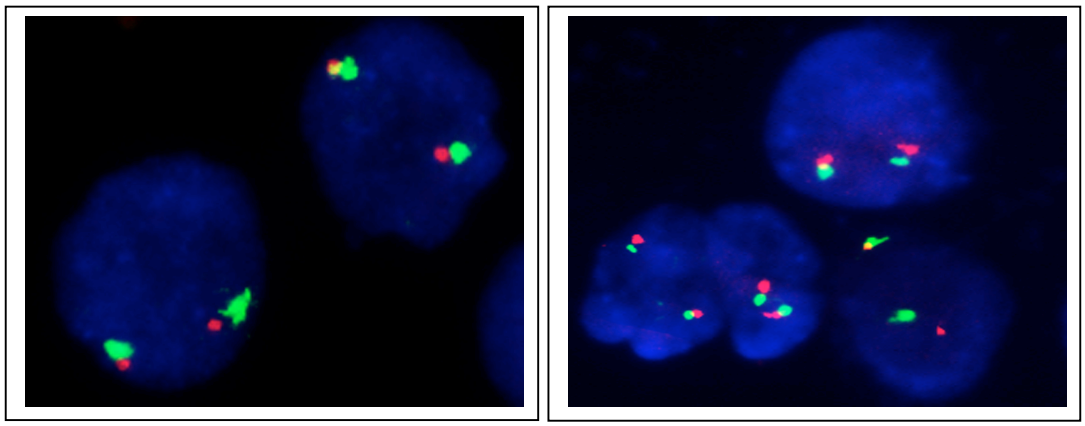
I. Sequencing Results

A single clone was selected for PCR to amplify the region with genetic mutation; PCR products were cloned into vectors for sequencing.



*** Introduction of two nucleotide mutations without alteration of protein sequence. GAA->GAG, amino acid E->E; GGT->GGA, amino acid G->G.**

II. FISH Detection of EGFR



Wild type SL702 ΔE746-A750/+

Figure 1. FISH detection of EGFR in wild type (WT) and SL702 cell lines. GeneCopoeia VividFISH™ FISH probes **CEP7/EGFR** were hybridized in WT and SL702. The green fluorescence indicates centromeres on chromosome 7, the orange fluorescence indicates the EGFR genes on chromosome 7. The cell nuclei are counter-stained with DAPI (blue).

Citation of product: If use of this item results in a publication, please use this information: HCT116/EGFR ΔE746-A750 cancer marker mutation cell lines (SL702, GeneCopoeia, Inc., Rockville, MD).

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