OncoSpot™ EGFR L858R Homozygous HCT116 Cancer Biomarker Mutant Cell Line

Catalog number: SL725

Product: EGFR L858R Homozygous HCT116 Cancer Biomarker mutant Cell Line

Description: This product is a HCT116 cell line genetically modified using CRISPR to have two alleles with L858R mutation of EGFR and puromycin marker in intron 18 of EGFR for selection. This cell line can be used in vitro as a reference for gene mutation detection or other related applications.

AA mutation: L->R

CDS mutation: C. 2573

Genotype: EGFR (L858R, Puro/L858R, Puro)

Genomic Mutation: CTG ->CGG

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 °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 °C 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 x g. 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 1 µg/mL

**Culture temperature:**

37 ºC with 5% CO2

**Subculture:**

Rinse the cells with PBS without cations, digest cells with 0.25% (w/v) Trypsin-EDTA (0.53 mM) solution and split at 1 x 10⁵ viable cells/mL to 1 x 10⁶ cells/mL.

**Cryopreservation**

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

**Wild type**

![Wild type sequence graph]

**Mutant (L858R: CTG to CGG)**

![Mutant sequence graph]
Citation of product: If use of this item results in a publication, please use this information:
HCT116/EGFR L858R homozygous cancer marker mutation cell lines (SL725, GeneCopoeia, Inc., Rockville, MD).

Limited Use License

A limited use license is granted to the Buyer of the Product. The Product shall be used by the Buyer for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Copyright ©2019 GeneCopoeia, Inc.

ONCO-SL-07082019

GeneCopoeia, Inc.
9620 Medical Center Drive, #101
Rockville, MD 20850 USA
Tel: 301-762-0888; Fax: 301-762-3888
Email: support@genecopoeia.com
Web: www.genecopoeia.com