

## OncoSpot<sup>™</sup> KRAS G13D Heterozygous HCT116 Cancer Biomarker Mutant Cell Line

Product:KRAS G13D Heterozygous HCT116 Cancer Biomarker mutant Cell LineDescription:This product is a HCT116 cell line genetically modified using CRISPR to have alleles with G13D mutation of KRAS and puromycin marker in intron 1 of K for selection, and the other allele with a wild type KRAS. This cell line can be u in vitro as a reference for gene mutation detection or other related application	RAS sed
alleles with G13D mutation of KRAS and puromycin marker in intron 1 of K for selection, and the other allele with a wild type KRAS. This cell line can be u	RAS sed
AA mutation: G->D	
CDS mutation: C. 38	
Genotype: KRAS (G13D, Puro/+)	
Genomic Mutation: GGC->GAC	
<b>Quantity:</b> 1 vial of 2 x 10 <sup>6</sup> cells; frozen	
Shipping conditions: Dry ice	
<b>Storage conditions:</b> Liquid nitrogen vapor phase. Remove the item from the dry ice packaging check all items for damage and leakage. Place immediately into storage a below -140 <sup>o</sup> C, preferably into the liquid nitrogen vapor phase, until use.	
Source of parental line: HCT116 Organism: <i>Homo sapiens,</i> 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.	
<b>Safety instructions:</b> 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 stora. The liquid nitrogen will be converted to gas upon thawing. Due to the nature on itrogen 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 0.6  $\mu$ g/mL

#### **Culture temperature:**

37 °C with 5% CO<sub>2</sub>

#### Subculture:

Rinse the cells with PBS without cations, digest cells with 0.25% (w/v) Trypsin-EDTA (0.53 mM) solution and split at  $1 \times 10^5$  viable cells/mL to  $1 \times 10^6$  cells/mL.

#### Cryopreservation

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

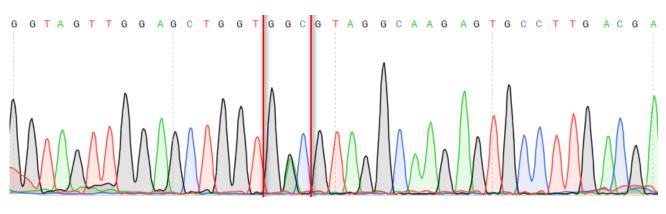


# **Product Quality Control:**

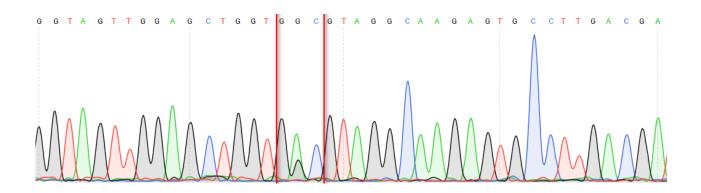
### I. Sequencing Results

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

Wild type



Mutant (G13D: GGCto GGC/GAC heterozygote)





If use of this item results in a publication, please use this information: HCT116/KRAS G13D heterozygous cancer biomarker mutant cell lines (SL742, GeneCopoeia, Inc., Rockville, MD).

#### **Limited Use License**

Citation of product:

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