Datasheet for HCT116-CBH-Cas9-HYG- AAVS1 Cell Line

Catalog number: SL572

Product: HCT116 cell line stably expressing CRISPR Cas9 nuclease

Description: This product is a cell line stably expressing the CRISPR Cas9 nuclease. Cas9 is integrated at the human AAVS1 Safe Harbor locus (also known as PPP1R2C). This cell line also expresses copGFP and the hygromycin resistance gene. In combination with separately transfected or transduced single guide RNAs (sgRNAs), this cell line will sustain double-strand DNA breaks (DSBs) at targeted genome sites. This cell line can be used *in vitro* for gene knockout, transgene knockin, mutagenesis, transgene integration, or other genome editing-related applications

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.

Transgene integration:

Source of parental line:

HCT116
Organism: *Homo sapiens*, human
Tissue: Colon
Disease: colorectal Carcinoma
Cell type: Epithelial

Quality control: >95% viability before freezing. All cells were tested and found to be free of mycoplasma, bacterial, 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 ^{0}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 125 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 RPMI 1640 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
Hygromycin to a final concentration of 300 µg/mL

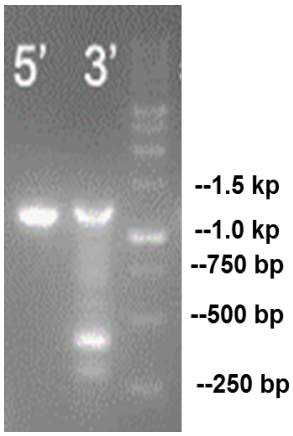
Culture temperature:
37 ^{0}C with 5% CO_2

Subculture
Replace culture medium with selection-free medium and incubate for up to 6 hours. Rinse the cells with PBS, digest cells with 0.25% (w/v) Trypsin-EDTA (0.53 mM) solution and split at 1:3 to 1:10 ratio.

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

QC Data:

1. Cas9 gene integration at AAVS1 site in HCT116-CBH/Cas9 cell line by Junctional PCR from genomic DNA



5′ '-Junctional PCR: predicted size 1.1 Kb

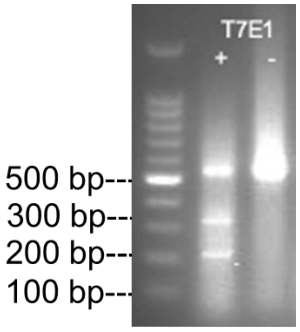
One primer is at the upstream region of the 5' homology arm, the other primer at the Cas9-plasmid region.

3′ '-Junctional PCR: predicted size 1.2 Kb

One primer is at the downstream region of the 3' homology arm region, the other primer is at the Cas9 plasmid region.

2. HCT116-CBH/Cas9 Activity by T7 Endonuclease I assay (T7E1)

T7E1-(T7Endonuclease1)

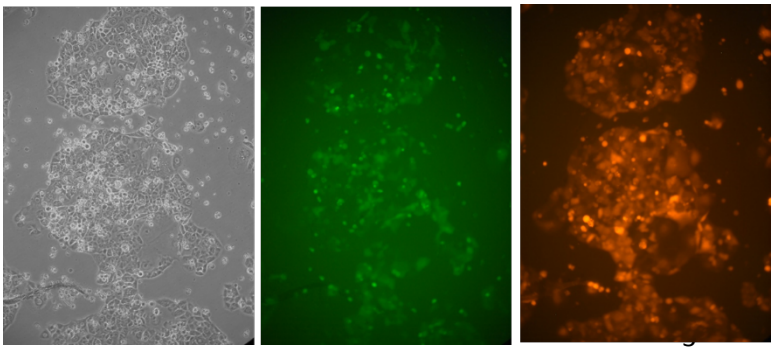


sgRNA targeting HUWE gene was transduced into HCT116- Cas9-hyg Stable Cell Line by transduction. HUWE gene was cut by Cas9 expressed inside the cells and repaired through NHEJ with mutation. A 525 bp HUWE gene fragment from PCR was then tested by T7 Endonuclease I (T7 E1) Assay. The T7 E1 cleavage will result in two additional bands:

one band at ~333bp and the other at ~192 bp.

3. Fluorescence images of HCT166/Cas9-HYG-Huwe-RFP cells

Light **eGFP** **RFP**



The HCT166/Cas9-HYG cells were transduced with Huwe-sgRNA-RFP lentivirus. The expression of eGFP and RFP were measured after 72hrs of transduction

Citation of product: If use of this item results in a publication, please use this information: CRISPR Cas9 stable HCT116 cell line (SL572; GeneCopoeia, Inc., Rockville, MD).

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