

## Datasheet for HEK293/HF-Cas9- AAVS1 Cell Line

**Catalog number:** SL553

**Product:** HEK293 cell line stably expressing CRISPR SpCas9-HF nuclease

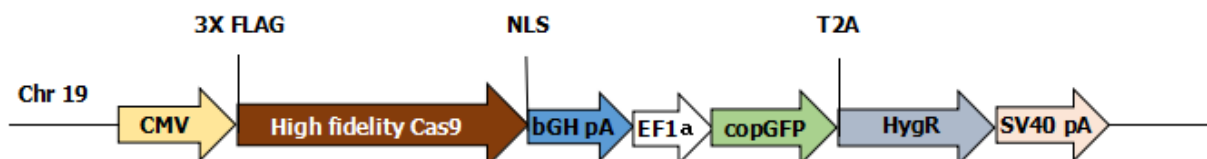
**Description:** This product is a cell line stably expressing the CRISPR SpCas9-HF1 Cas9 nuclease, a high-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. SpCas9-HF1 Cas9 is integrated at the human AAVS1 Safe Harbor locus (also known as PPP1R2C). This cell line also 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 knock-in, mutagenesis, transgene integration, or other genome editing-related applications

**Quantity:** 1 vial of  $2 \times 10^6$  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  $-140^{\circ}\text{C}$ , preferably into the liquid nitrogen vapor phase, until use.

**Transgene integration:**



**Source of parental line:**

HEK293  
Organism: *Homo sapiens*, human  
Tissue: Embryonic kidney  
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 °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 is Dulbecco's Modified Eagle's Medium (DMEM). 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**

Hgromycin to a final concentration of 150 µg/mL

**Culture temperature:**

37 °C with 5% CO<sub>2</sub>

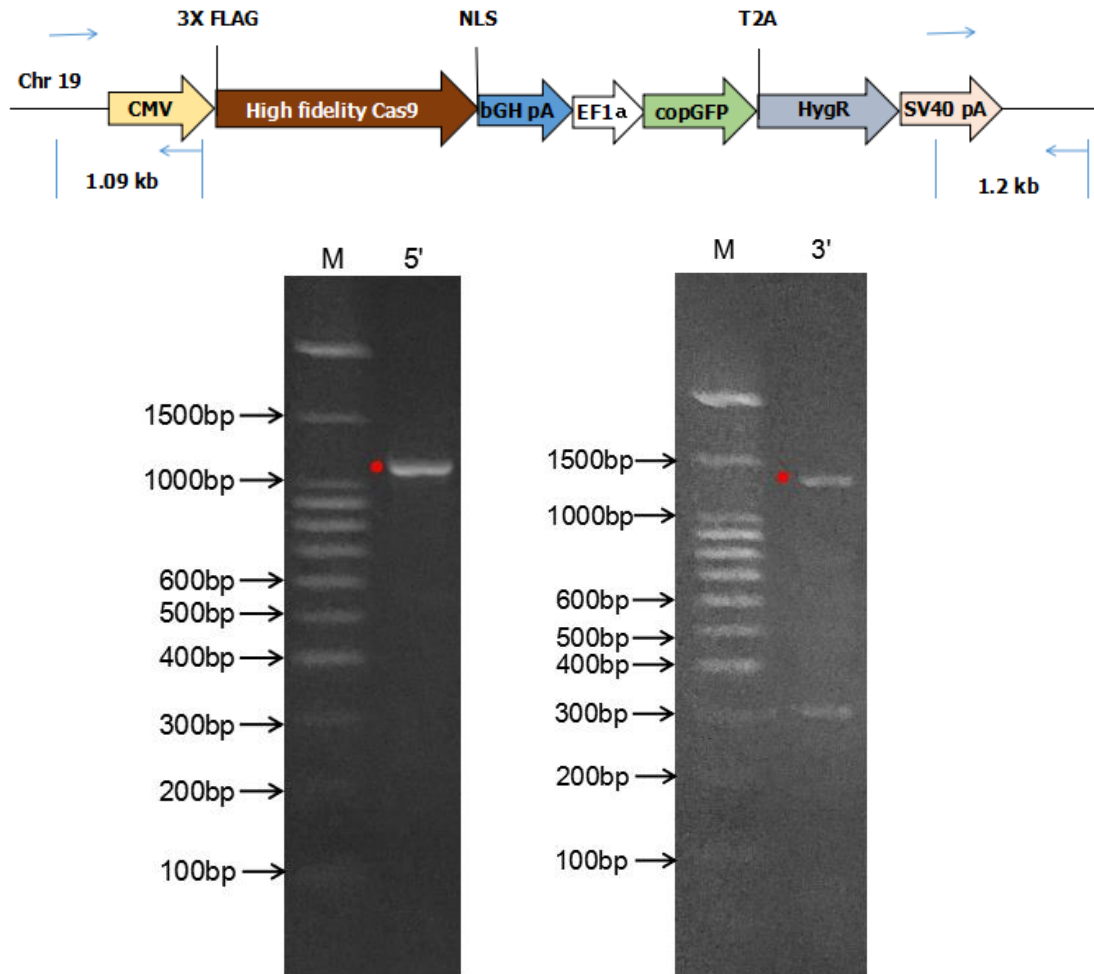
**Subculture:**

Replace culture medium with selection-free medium and incubate for up to 6 hours. Rinse the cells with PBS without cations, digest cells with 0.25% (w/v) Trypsin-EDTA (0.53 mM) solution and split at 1:6 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 HEK293/HF-Cas9 cell line by Junction PCR from genomic DNA**



5'-Junction PCR size was 1092 bp. One primer recognizes the chromosome outside of the 5' homology arm region; the other primer recognizes the Cas9-plasmid region.

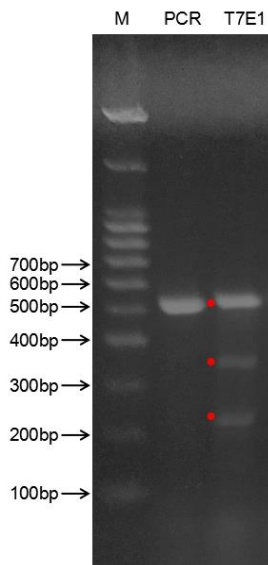
3'-Junction PCR size was 1257 bp. One primer recognizes the chromosome outside of the 3' homology arm region; the other primer recognizes the Cas9-plasmid region.

## 2. HEK293/Cas9 Activity by T7 Endonuclease I assay (T7E1)

A lentivirus expressing sgRNA targeted to the HUWE gene was transduced into HEK293 / Cas9-HF1 hyg stable cell line. The 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 results in two additional bands: one ~192 bp and the other ~333 bp.

HUWE-F:AAGGGTGGGACGTGAACTTGTC

HUWE-R:AGAATCTCCCATCAACCCT



**Citation of product:** If use of this item results in a publication, please use this information: CRISPR SpCas9-HF Cas9 stable HEK293 cell line (SL553; GeneCopoeia, Inc., Rockville, MD).

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