

Datasheet for HEK293/LoxP-Cas9-hyg-AAVS1 Cell Line

Catalog number: SL554

Product: HEK293 cell line stably expressing CRISPR Cas9 nuclease from AAVS1 locus.

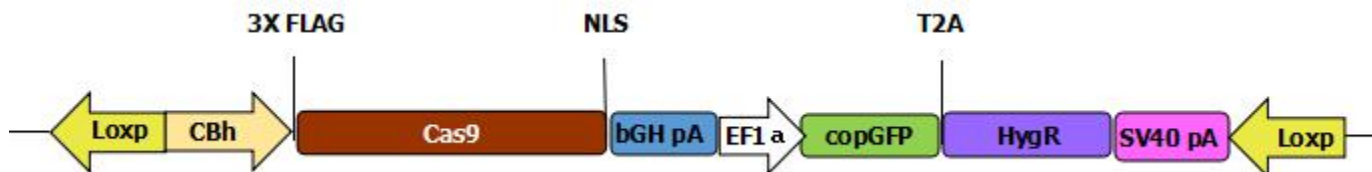
Description: This product is a cell line stably expressing the CRISPR Cas9 nuclease. Cas9 is integrated at the human AAVS1 Safe Harbor locus. This cell line also expresses copGFP and the hygromycin resistance gene. In combination with separately transfected or transduced single guide RNAs (sgRNAs), this 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. The Cas9 and selection cassettes are flanked by LoxP sites and thus can be removed using a transient expression of Cre recombinase or transfection of Cre protein. Negative TK gene is used to remove random integration.

Quantity: 1 vial of 2×10^6 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 $-140\text{ }^{\circ}\text{C}$, 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, 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 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 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

Hygromycin to a final concentration of 300 µg/mL

Culture temperature:

37 °C with 5% CO₂

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.

Mycoplasma: Negative
(MycoAlert Mycoplasma Detection Kit from Lonza)

Product QC:

1. Junctional PCR (to confirm the Cas9 gene integration into AAVS1 site)

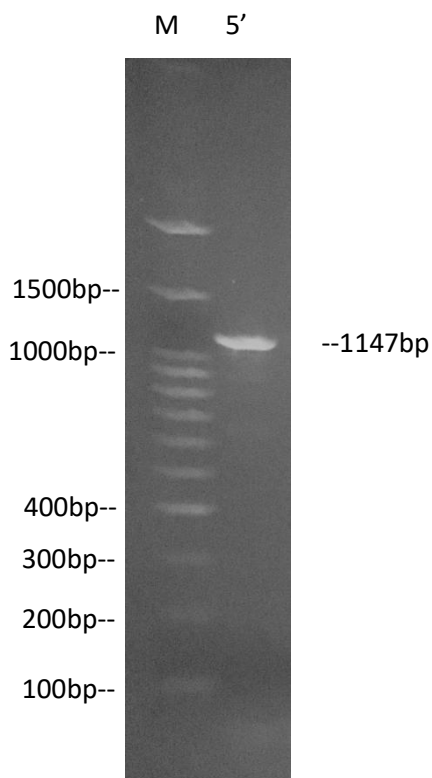
(1) 5' Junctional PCR

one primer from chromosomal outside of the 5' homology arm region, the other primer from the Cas9-plasmid region to confirm the AAVS1 site integration of Cas9 expressing cassette

Junction-PCR 5'F: GGCGGCCTTAATTAAGCGAATTC

Junction-PCR 5'R: GCGTACTTGGCATATGAT

Predict product length: 1147 bp



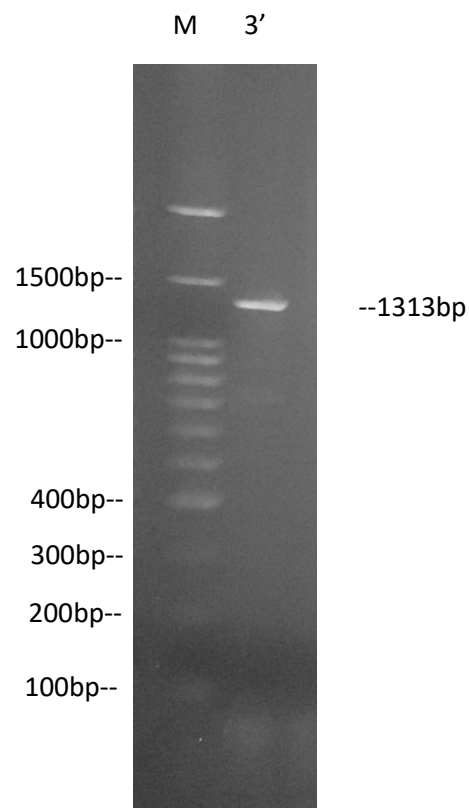
(2) 3' Junctional PCR to confirm 3' integration

one primer from chromosomal outside of the 3' homology arm region, the other primer from the Cas9-plasmid region to confirm the AAVS1 site integration of Cas9 expressing cassette

Junction-PCR 3'F: GTTAACTTGTTTATTGCAGCTTATAATGG

Junction-PCR 3'R: GAATTGATTAAATGCTGTCGAC

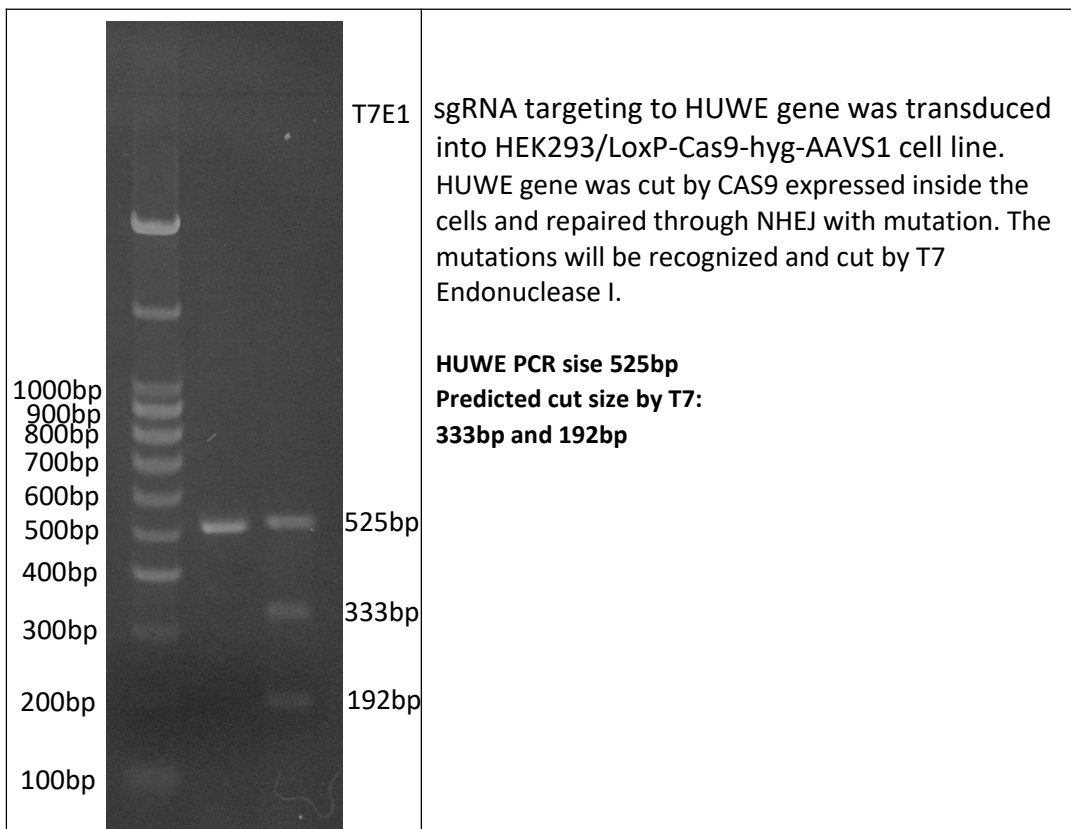
Predict product length: 1313 bp



2. T7 Endonuclease I (T7 E1) Assay

HUWE Site T7 E1 Assay

sgRNA targeting to human HUWE gene was transfected into HEK293/LoxP-Cas9-hyg-AAVS1 cell line by transient transfection. After transfection, HUWE gene was cut by CAS9 expressed inside the cells and repaired through NHEJ with mutation. A 525bp 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 ~192bp and the other ~333bp.

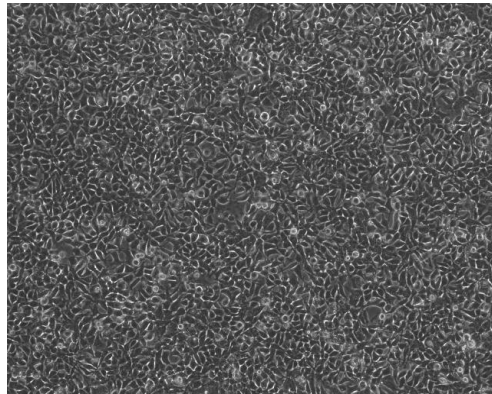
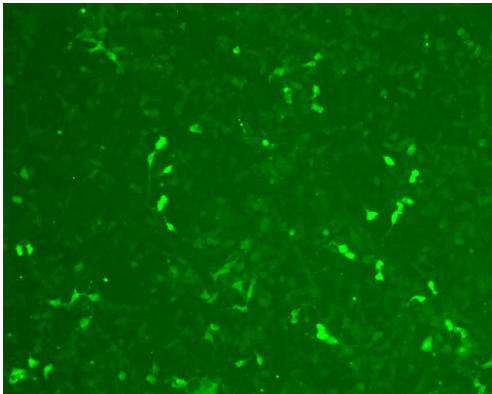


3.Cre recombinase inducible conditional gene knockout

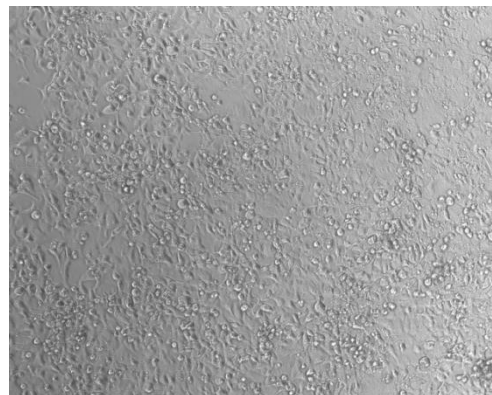
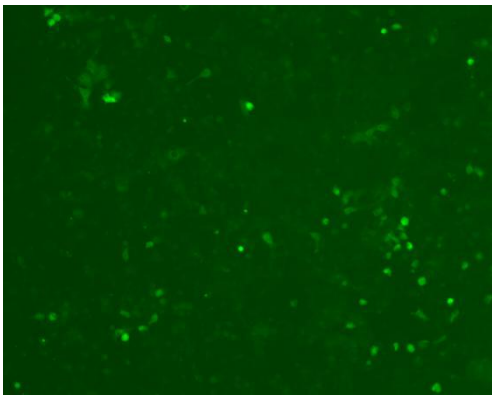
The Cas9 and selection cassettes are flanked by LoxP sites and thus can be removed using a transient expression of Cre recombinase or transfection of Cre protein. After transfection, CRE recombinase will recognize the LoxP sites and cut the gene between the two sites. So the fluorescence will decrease or reduce under fluorescence microscope.

After transient transfection 72hrs:

Exposure time: 2s(fluorescence); 1ms(bright)



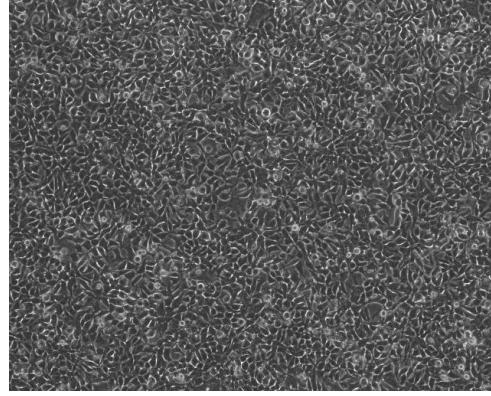
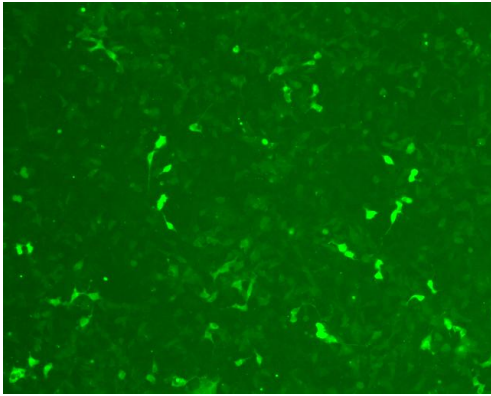
Single clone



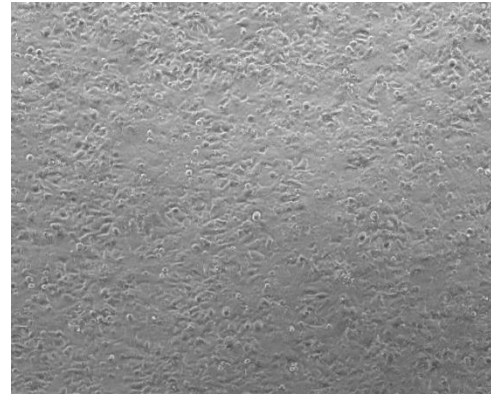
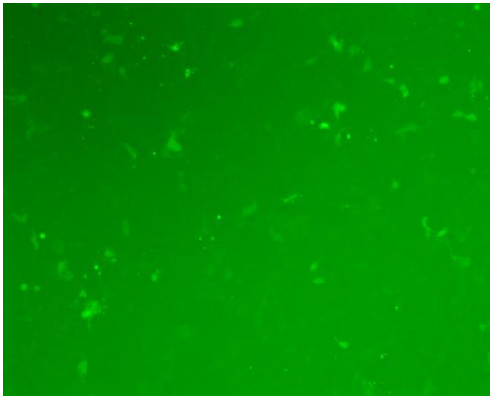
Single clone transiently transfected with CRE

After lentivirus transduction 72hrs:

Exposure time: 2s(fluorescence); 1ms(bright)



Single clone



Single clone transduced with CRE lentivirus

Citation of product: If use of this item results in a publication, please use this information:
CRISPR Cas9 stable HEK293/LoxP-Cas9-hyg-AAVS1 (SL554, GeneCopoeia, Inc.,
Rockville, MD).

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