Datasheet for HEK293/Inducible Cas9 Safe Harbor Cell Line

Catalog number: SL507

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

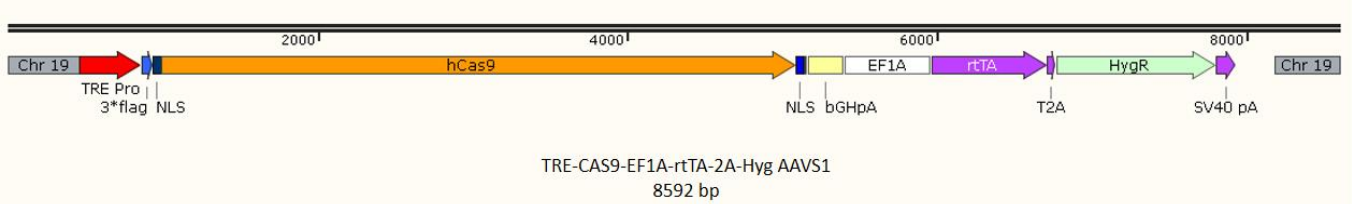
Description: This product is a cell line stably expressing the CRISPR Cas9 nuclease. Cas9 is integrated at the human AAVS1 Safe Harbor locus (also known as PPP1R2C). This cell line also expresses rtTA regulating protein and the hygromycin resistance gene. In combination with separately transfected or transduced single guide RNAs (sgRNAs), this cell line will sustain double-strand DNA breaks (DSBs) at targeted genome sites after Dox induction. Though we could not detect Cas9 expression before Dox induction by Western blot, we found the basal Cas9 activity by T7 Endonuclease I assay before Dox induction when sgRNA was expressed at high levels using plasmid transfection. Nonetheless, the controlled Cas9 expression level by Dox induction can reduce the off-target effects of the sgRNA. 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℃, preferably into the liquid nitrogen vapor phase, until use.

Transgene integration:



TRE-CAS9-EF1A-rtTA-2A-Hyg AAVS1
8592 bp

Source of parental line:

HEK293
Organism: *Homo sapiens*,
human Tissue: 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℃ 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: dialyzed fetal bovine serum to a final concentration of 10%.

Selection

Hygromycin to a final concentration of 50 µg/mL

Cas9 induction: 1 ug/ml Doxycycline

Culture temperature:

37℃ with 5% CO_2

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 1:3 to 1:10 ratio.

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

Mycoplasma: Negative
(MycoAllert Mycoplasma Detection Kit from Lonza)

Product QC:

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

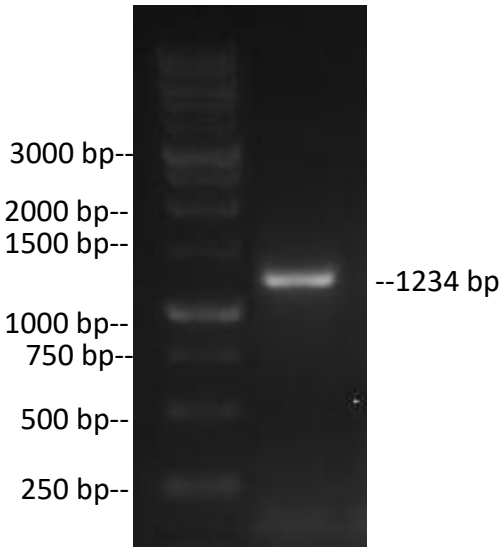
(1) 5' Junctional PCR

One primer recognizes the chromosome 19 region outside of the 5' homology arm region, while the other primer recognizes the Cas9-plasmid region to confirm the AAVS1 site integration of the Cas9 expressing cassette

Junction-PCR 5'F: CCGGAACTCTGCCCTCTAAC

Junction-PCR 5'R: AGGCGATCTGACGGTTCACT

Predict product length：1234 bp



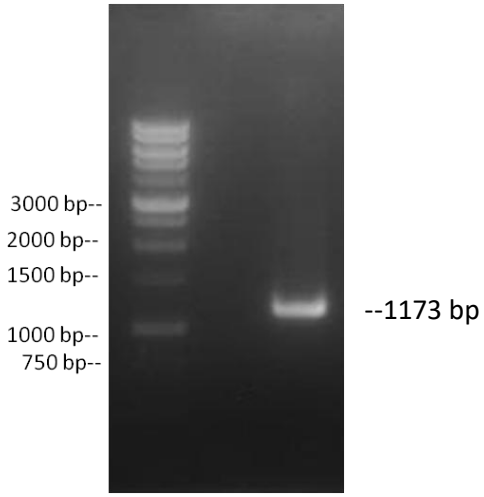
(2) 3' Junction PCR to confirm 3' integration

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

Junction-PCR 3'F: CTTGCTCTGGTCAACCAGGT

Junction-PCR 3'R: TCCTGGGATACCCCGAAGAG

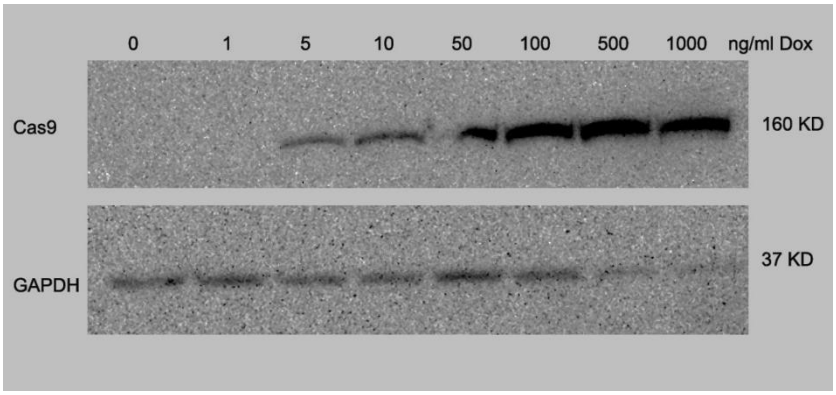
Predict product length：1173 bp



2. Western blot for Cas9 Expression after Dox induction for HEK293/inducible Cas9-Hyg-AAVS1 Cell Line

HEK293/inducible Cas9-Hyg-AAVS1 cells were induced by different concentration of Dox for 40 hours before Western blot analysis.

Western blot using anti-Cas9 mAb (Abcam ab191468) at 5 ug/ml.

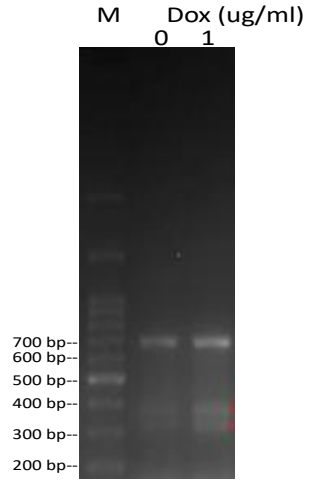


The Cas9 expression could not be detected by Cas9 mAb for uninduced control and the induction at 1 ng/ul Dox. The Cas9 expression is Dox dose dependent.

3. T7 Endonuclease I (T7 E1) Assay

(1) EMX1 Site T7 E1 Assay

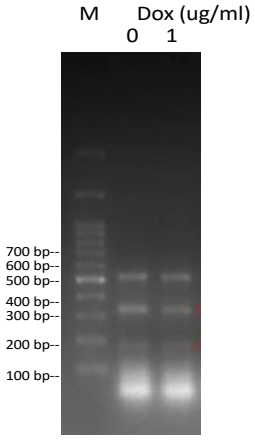
sgRNA targeting to EMX1 gene was transduced into HEK293/Inducible CAS9 AAVS1 cell line by transient transfection. After transfection, Cas9 protein was induced for 72 hours by 1 ug/ml Dox. EMX1 gene was cut by CAS9 expressed inside the cells and repaired through NHEJ with mutation. A 684 bp HUWE gene fragment from PCR was then tested by T7 Endonuclease I (T7 E1) Assay. The T7 E1 cleavage results in two additional bands: one ~315 bp and the other ~369 bp.



(2) HUWE Site T7 E1 Assay

sgRNA targeting to HUWE gene was transduced into HEK293/Inducible CAS9 AAVS1 cell line by transient transfection. After transfection, Cas9 protein was induced for 72 hours by 1 ug/ml Dox. 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.



Citation of product: If use of this item results in a publication, please use this information: CRISPR Cas9 stable HEK293/inducible Cas9 AAVS1 cell line (SL507, GeneCopoeia, Inc., Rockville, MD).

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