# Datasheet for HCC827/ Cas9-hyg Stable Cell Line

**Catalog number:** SL578

**Product:** HCC827 cell line stably expressing CRISPR Cas9 nuclease

**Description:** This product is a cell line stably expressing CRISPR Cas9 nuclease. The cell line also expresses 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 for in vitro 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:**

HCC827

| | |
|---|---|
| Organism: | Homo sapiens, human |
| Human Tissue: | Lung |
| Disease: | adenocarcinoma |
| Cell type: | Epithelial |

**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 before transferring the vial into cell culture hood. Using aseptic technique, add the contents of the vial to 9 ml of complete growth medium (without selection), centrifuge for 5 minutes at 250 x g to remove the cryoprotective medium. Resuspend the cell pellet 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 RPMI. 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 100 µg/mL

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

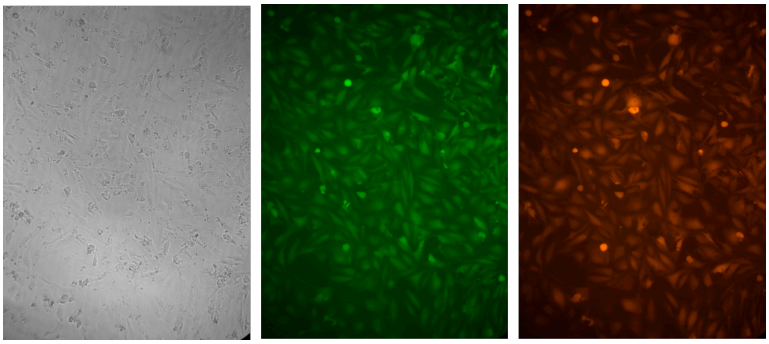
**Subculture**
Rinse the cells with PBS without cations, digest cells with 0.25% (w/v) Trypsin-EDTA (0.53 mM) solution and split at 1:2 to 1:4 ratio.

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

**Product QC:** >95% viability before freezing. All cells were tested and found to be free of mycoplasma, bacterial, viruses, and other toxins.

## Cas9 Activity Testing by T7 Endonuclease I (T7E1) Assay

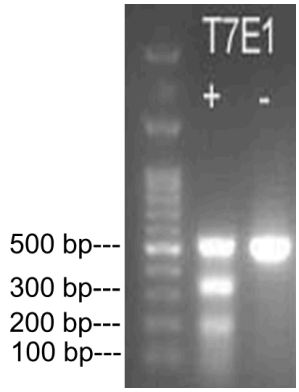
### Fluorescence images of HCC827/Cas9-HYG-Huwe-RFP cells



**light** **eGFP** **RFP**

HCC827/Cas9-HYG cells were transduced with Huwe-sgRNA-RFP lentivirus. The expression of eGFP and RFP were measured after 72hrs of transduction

### T7E1 (T7 Endonuclease 1)




sgRNA targeting HUWE gene was transduced into HCC827-/Cas9-hyg Stable Cell Line by transduction. HUWE gene was cut by Cas9 expressed inside the cells and repaired through NHEJ with mutation. A 520 bp HUWE gene fragment from PCR was then tested by T7 Endonuclease I (T7 E1) Assay.

The uncut HUWE gene fragment is 520 bp. The T7 E1 cleavage will result in two additional bands: one ~329bp and the other ~191 bp

**Citation of product:** If use of this item results in a publication, please use this information: CRISPR Cas9 HCC827-Cas9-hyg Stable cell line (SL578, GeneCopoeia, Inc., Rockville, MD).

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