

Datasheet for RKO/ Cas9-hyg Stable Cell Line

Catalog number: SL539

Product: RKO cell line stably expressing CRISPR Cas9 nuclease

Description: This product is a cell line stably expressing the CRISPR Cas9 nuclease. This 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. We validated Cas9 activity in this cell line using the T7 Endonuclease I assay. 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 2×10^6 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°C , preferably into the liquid nitrogen vapor phase, until use.

Transgene integration:



Source of parental line:

RKO
Organism: Homo sapiens, human
Tissue: colon
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 250 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 RPMI1640. 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 400 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 1:3 to 1:12 ratio.

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

Mycoplasma: Negative
(MycoAlert Mycoplasma Detection Kit from Lonza)

Product QC:

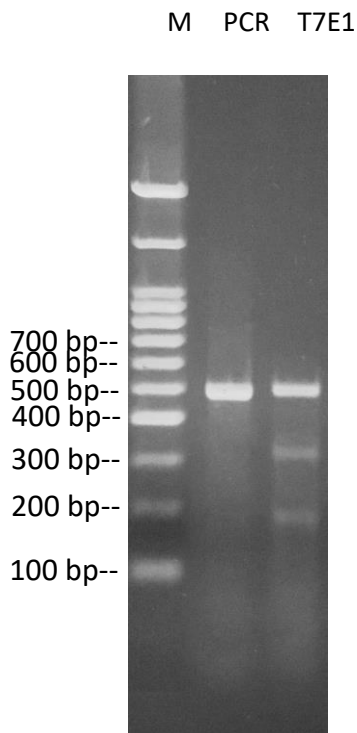
T7 Endonuclease I (T7 E1) Assay

HUWE Site T7 E1 Assay

A lentivirus expressing sgRNA targeted to the HUWE gene was transduced into RKO / Cas9-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:AGAATCTTCCCATCAACCCT



Citation of product: If use of this item results in a publication, please use this information:
CRISPR Cas9 RKO/ Cas9-hyg Stable cell line (SL539, GeneCopoeia, Inc., Rockville, MD).

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