Datasheet for hSMC / Cas9-hyg Stable Cell Line

Catalog number: SL586

Product: hSMC cell line stably expressing CRISPR Cas9 nuclease

Description: This product is an immortalized 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 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 ^{0}C, preferably into the liquid nitrogen vapor phase, until use.

Transgene integration:

Source of parental line:

human Carotid Artery Smooth Muscle Cells

Organism:	Homo sapiens
Tissue:	Carotid Artery
Cell type:	Muscle

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 complete growth medium for this cell line is SmGMTM- 2 Smooth Muscle Cell Growth Medium(Lonza,CC-3182).

Selection

Hygromycin to a final concentration of 200 μg/mL

Culture temperature:

37 ^{0}C with 5% CO_2

Subculture:

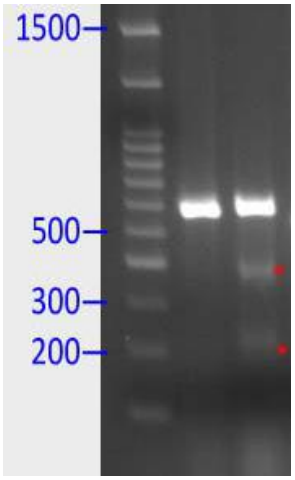
Rinse the cells with PBS without cations, digest cells with 0.25% (w/v) Trypsin-EDTA (0.53 mM) solution and split at 1:3 to 1:8 ratio.

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

TE1: T7E1 (T7 Endonuclease 1)



sgRNA targeting to EMX1 gene was transduced into hSMC/CAS9-Hyg cell line by lentivirus particals. EMX1 gene recognized by sgRNA was cut by CAS9 expressed inside the cells and repaired through NHEJ with mutations. The mutations will be recognized and cut by T7 Endonuclease I.

For EMX1 sgRNA transduced cells, a 579bp EMX1 gene fragment from PCR was tested by T7E1 Assay. The T7E1 cleavage will results in two additional bands:
211 bp and 368 bp.

Citation of product: If use of this item results in a publication, please use this information: CRISPR Cas9 hSMC-Cas9-hyg Stable cell line (SL586, GeneCopoeia, Inc., Rockville, MD).

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