

Datasheet for Mouse Neuro-2a/Inducible Cas9 Safe Harbor Cell Line

Catalog number: SL508

Product: Mouse Neuro-2A cell line stably expressing inducible CRISPR Cas9 nuclease from ROSA26 locus.

Description: This product is a cell line stably expressing the CRISPR Cas9 nuclease. Cas9 is integrated at the mouse Rosa26 Safe Harbor locus. 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 Doxycycline induction. This cell line can be used *in vitro* for gene knockout, transgene knockin, mutagenesis, transgene integration, or other genome editing-related applications.

The Tet3G inducible system has very tight control of leaking expression before induction by addition of doxycycline. In other cell lines, we have observed by Western blotting that Cas9 protein expression is undetectable in the absence of doxycycline and strongly induced in a doxycycline dose-dependent manner. However, we found the basal Cas9 activity before doxycycline induction is similar to that from doxycycline-induced Cas9 activity by T7 Endonuclease I assay when sgRNAs are expressed at high levels using plasmid transfection. In other cell lines, we have observed that reducing doxycycline levels reduces background Cas9 activity, especially when the sgRNA has been integrated at low copy using lentivirus. In addition, reducing Cas9 expression levels has been shown to reduce CRISPR off-target activity in mouse embryo pronuclear injection (Fujii, et al., 2013). Therefore, reducing the doxycycline dose might reduce the off-target effects of the sgRNA, especially when the sgRNA is integrated at low copy number using lentiviral transduction rather than DNA transfection.

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:

Neuro-2A
Organism: *Mus musculus*, mouse

Tissue: Brain

Cell type: Neuroblast

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: dialyzed fetal bovine serum to a final concentration of 10%

Selection:

Hygromycin to a final concentration of 100 µg/mL

Cas9 induction: 1 ug/ml Doxycycline

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 1:10 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. Junction PCR (to confirm the Cas9 gene integration into ROSA26 site)

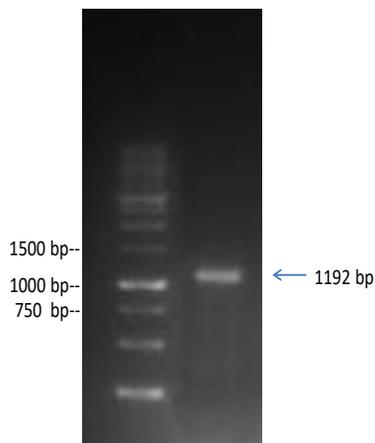
(1) 5' Junction PCR

One primer recognizes the region of the chromosome outside of the 5' homology arm region, while the other primer recognizes the Cas9-plasmid region to confirm the ROSA26 site integration of the Cas9 expressing cassette

Junction-PCR 5'F: CTCGTCGCTGATTGGCTTCT

Junction-PCR 5'R: AGGCGATCTGACGGTTCCT

Predict product length : 1192 bp



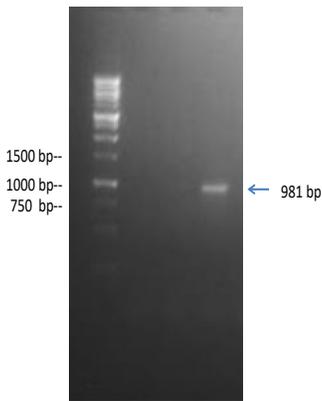
(2) 3' Junction PCR to confirm 3' integration

One primer recognizes the region of the chromosome outside of the 5' homology arm region, while the other primer recognizes the Cas9-plasmid region to confirm the ROSA26 site integration of the Cas9 expressing cassette

Junction-PCR 3'F: CTTGCTCTGGTCAACCAGGT

Junction-PCR 3'R: GGAGACATCCACCTGGAAACC

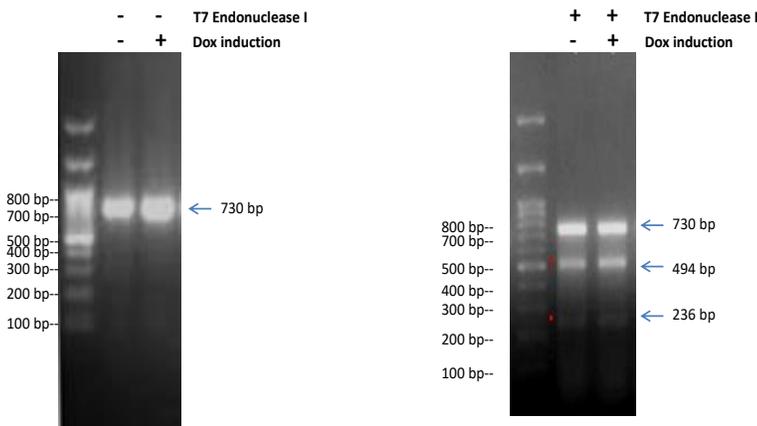
Predict product length : 981 bp



2. T7 Endonuclease I (T7 E1) Assay

(1) P2X2r Site T7 E1 Assay

An sgRNA targeted to the mouse P2X2r gene was transduced into the Neuro-2A/Inducible CAS9 ROSA26 cell line by transient transfection. After transfection, Cas9 protein was induced for 72 hours with 1 ug/ml Doxycycline. The P2rX2 gene was cut by Cas9 expressed inside the cells and repaired through NHEJ, causing an indel mutation. Using the GeneCopoeia IndelCheck™ insertion/deletion detection system, a 730 bp P2X2r gene fragment from the PCR was then digested with T7 Endonuclease I (T7 E1). T7 E1 cleavage results in two additional bands: one ~494 bp and the other ~236 bp.



Left: P2X2r gene PCR without T7 E1, Dox uninduced or induced.

Right: P2X2r gene PCR with T7 E1, Dox uninduced or induced. The uninduced cells still express enough Cas9 for P2X2r sgRNA plasmid to cut the P2X2r gene. It has been reported that high copies of sgRNA molecules will only need minimal amount of Cas9 protein in CRISPR assay.

References

Fujii, et al. (2013). Efficient generation of large-scale genome-modified mice using gRNA and CAS9 endonuclease. *Nucleic Acids Research* 41, e187.

Citation of product: If use of this item results in a publication, please use this information: CRISPR Cas9 stable Neuro-2A/inducible Cas9 ROSA26 cell line (SL505, GeneCopoeia, Inc., Rockville, MD).

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