Applied StemCell, Inc.
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Datasheet

mESC-C9m (Cas9 expressing mouse embryonic stem cells)

Order information

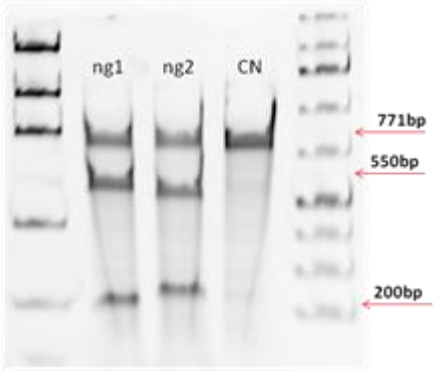
Catalog Number	Quantity
ASE-9311	5.0 x 10^5 cells/ vial

Product Information

Description

Guide RNA (gRNA) validation for embryo microinjection is usually performed in easy-to-transfect cell lines, such as N2A cells. The gRNA activity validated in these cell lines however does not always reflect what really happens in embryos. Mouse embryonic stem cells of the C57BL6 strain, which resemble the closest to mouse embryos, were engineered to stably express the S. pyogenes Cas9 nuclease. This cell line can be used for gRNA validation by transfecting the gRNA into the cell line followed by mismatch assay.

The data below indicate the validation result of two guide RNAs. The mESC-C9m cells were transfected with two oligo-synthesized guide RNAs. Genomic DNA was harvested 72 hours after transfection. Both guide RNAs are shown active in the mismatch assay.



ng1 cleavage 207/564bp
ng2 cleavage 237/534bp
CN: control no cleavage

Shipping

Dry ice

Storage and Stability

Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.

Quality Control

To ensure the expression of Cas9, the cell line should be periodically cultured in hygromycin 100µg/mL. The positive control guide RNA, g1 or g2, can be used as a control for gRNA validation assay.

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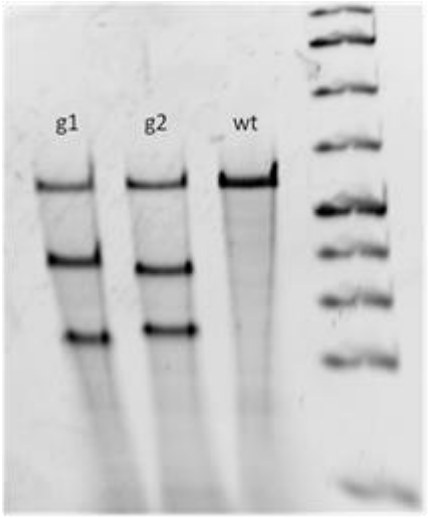

g1 cleavages 234/379bp
g2 cleavages 257/356bp

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Protocol

1. Cell culture

Media Component	Final Conc.
Knockout DMEM (Thermo Fisher)	
Knockout Serum Replacement (Thermo Fisher)	15%
Sodium Pyruvate (Thermo Fisher)	1 mM
NEAA (Thermo Fisher)	0.I mM
2-Mercaptoethanol (Sigma)	0.1 mM
mLIF (Milipore 10^7 U/mL)	1000 U/mL
Human Insulin (Sigma)	0.0005%
PD032591 (LC Lab)	1 µM
CHIR99021 (LC Lab)	3 µM
Forskolin (LC Lab)	10 µM
GlutaMAX™	1 mM

2. Guide RNA preparation

2.1 Oligo-synthesis: Order synthetic guide RNA

2.2 *In vitro* transcription: Use HiScribe T7 High Yield RNA Synthesis Kit (NEB, Cat. #: E2040S) to transcribe gRNAs.

2.2.1 Clean the lab bench and pipetors by wiping with RNase*Zap*® RNase Decontamination Solution.

2.2.2 Keep the T7 RNA Polymerase Mix on ice.

2.2.3 Thaw the T7 10X Reaction Buffer, four ribonucleotide solutions, and ddH_2O at room temperature.
- Briefly vortex the T7 10X Reaction Buffer and ribonucleotide solutions. Centrifuge before open tubes.

2.2.4 Prepare template:
- HiScribe T7 High Yield RNA Synthesis Kit suggests 1 µg template DNA for short RNA transcription (0.3 kb).
- Ambion MEGAshortscript Kit suggests 260 ng DNA templates in 20 µL reaction to yield best amount of RNA (~90 µg) from 100 bp-length DNA.

2.2.5 Assemble the reaction mix in an RNase-free microfuge tube at room temperature as shown below.

2.2.6 dNTPs, 10X Buffer and Enzyme Mix can be premixed as 9 µL mixture added into 20 µL reaction:
- For short RNA transcripts (0.3 kb)

Segments	Volume (µL)
T7 10X Reaction Buffer	1.5
dNTPs mix (75 mM)	1.5 X 4
T7 Enzyme Mix	1.5
Template DNAs (100 ng - 1 µg)	11

2.2.7 Mix contents thoroughly by gently flicking the tube.

2.2.8 Incubate the reaction at 37°C for at least 4 hrs.

3. Transfection: Lipofectamine

3.1 Plate 1 x 10^5 cells/mL density of cells with fresh medium in one well of 12-well plate the day before transfection.

3.2 Add 2.5µL of Dharmacon gRNA plus 2.5µL of Dharmacon rRNA to 50µL OPTI-MEM and add 3µL Lipofectamin 3000 to 50µL OPTI-MEM. Mix together for 5 min.

3.3 Add 2µg IVT gRNA to 50µL OPTI-MEM and add 3µL Lipofectamin 3000 to 50µL OPTI-MEM. Mix together for 5 min.

3.4 Add 100µL transfection solution to cells

3.5 Collect the transfected cells 72 hours after transfection for mismatch essay. Refresh the medium if necessary during the culture.

4. Mismatch Assay

4.1 Extract genomic DNA.

4.2 PCR 100ng of genomic DNA using 2x Fusion (Fisher, Cat #: F548L)

4.3 Run 2µL of PCR sample on 2% agarose gel with Low Mass ladder (Invitrogen, Cat.#:10068-013) to estimate concentration.

4.4 Adjust concentration to 400ng in 17µL volume with diH2O.

4.5 Hybridize PCR sample to form heteroduplexes using program below in a thermocycler.

Temperature	Time	Temperature ramp
95°C	10 min	
95°C to 85°C		(-2.0°C/s)
85°C	1 min	
85°C to 75°C		(-0.3°C/s)
75°C	1 min	
75°C to 65°C		(-0.3°C/s)
65°C	1 min	
65°C to 55°C		(-0.3°C/s)
55°C	1 min	
55°C to 45°C		(-0.3°C/s)
45°C	1 min	

45°C to 35°C		(-0.3°C/s)
35°C	1 min	
35°C to 25°C		(-0.3°C/s)
25°C	1 min	
4°C	Hold ∞	

4.6 Add 2µL of 10X NEBuffer 2 and 1µL of T7 Endonuclease I (NEB, cat. #M0302S)
4.7 Incubate at 37°C for 15min. Add 2µL of 0.5M EDTA to stop the reaction.
4.8 Run 16µL of sample on acrylamide gel.

5. Cryopreservation

Resuspend the cells in the culture medium with supplement of 10% DMSO and place vials into an isopropanol freezing container and place the container at -80°C overnight. Transfer to a liquid nitrogen tank on the following day.