Cell Use Instruction - IPSC-DYR0100-CAS9 Cell Line

Product Info

Catalog	YC-C098-Cas9-H		
Cell line	IPSC-DYR0100-CAS9	Morphology	Spherical clone
Fluorescent & resistance	No fluorescence，Hygro	Passage ratio	1:6-1:8
Culture method	EZ-Stem™ Cell Culture Medium Ubigene didn't use P/S. But client could use P/S after cells grow in good condition after thawing.		
Cryopreservation solution	EZ-Stem™ Cryopreservation Medium		
Antibiotic concentration for maintenance	H=50.0μg/mL Note: To maintain stable expression of the cas9 gene, maintain cells under antibiotic selection during optimal growth conditions.		
Special Note	1、Add 0.25μL EZ-Stem Apoptosis inhibitor Medium to 1mL EZ-Stem Complete Medium for cell thawing and passaging. 2、The culture medium for stem cells needs to be changed daily.		

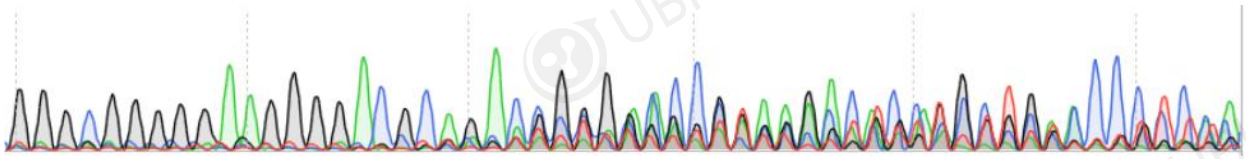
Product Validation Data

1) RT-QPCR

Sample Name	Target Name	Cт Mean	ΔCт
IPSC-DYR0100-CAS9	Cas9	21.07497406	7.93462086
IPSC-DYR0100-CAS9	β-actin	13.14035320	
IPSC-DYR0100	Cas9	34.49109268	21.41424656

IPSC-DYR0100	β-actin	13.07684612	

2) Cutting Efficiency Validation



Note: The above figure shows the sequencing peaks of the IPSC-DYR0100-CAS9 stable cell pool which is electroporated by ABCA gene targeting gRNA plasmid, after 48h antibiotic screening. The red arrow indicates the position where the nested peak appears, which shows that the genotype of the target site is significantly changed due to the cutting. Therefore, it indicates that Cas9 nuclease is successfully expressed.

Use of Cas9 stable cell line

1) The cell line stably expresses Cas9 nuclease. Gene knockout can be achieved by transfecting the gRNA into the Cas9 stable cell line. Gene knock-in and point mutation can be achieved by transfecting the gRNA and Donor DNA.
2) The transfected gRNA can be the form of plasmid, synthetic or vitro transcribed sgRNA. The transfer method can be transient transfection (e.g. liposome method, or electrotransfer method), or stable transduction (such as lentivirus method).
3) Long term culture of cell line in vitro may lead to changes in cell genome. It could be some changes in the expression of Cas9 (expression decreases). Therefore, it is recommended to use cell lines with low number of passages (within 10 passages) for experiments.

Cell Reception

Cryopreserved cells:

In the case of cryopreserved cells transported with dry ice, upon received, immediately transfer to liquid nitrogen for storage or store briefly at -80°C freezer, or proceed directly to cell thawing. Upon cell thawing, please count the cell number and cell viability and take some photos of the cells under different magnification (e.g. at 100x and 40x) as the records.

Notice: Upon received, please ensure to takes photos of the package, including dry ice and the tubes, and contact us within 24 hrs if any abnormalities such as dry ice has ran out, the cap of the cryovial is dislodged, broken and the cell is contaminated.

Cell Thawing (Based on 3 wells of a 6-well plate)

1)Preparation:

Preheat the EZ-Stem Complete Medium at room temperature in advance. Take the cryopreserved vial from liquid nitrogen to a box filled with dry ice, and leave for several minutes to volatilize residual liquid nitrogen;

The 6-well plate needs to be coated with Matrigel, which must be dissolved at 4°C, and the coating process should be completed in a short time, because the Matrigel will solidify soon when temperature above 10°C. Prepare the solution following the ratio of the Matrigel versus DMEM/F12 basal medium=1:100, mix well and add 1ml of Matrigel solution to each well of a 6-well plate after mixing, shake to make the bottom surface be coverred evenly. It can be used after being placed at 37°C for two hours.

2)Take out the cryopreserved vial from dry ice, shake slightly before thawing to remove residual dry ice. Then hold the cap with forceps, quickly thaw cells in a 37°C water bath by gently swirling the vial

(Note: keep the cap out of the water). In about 1 minute, it would completely thaw; Inside the ultra-clean bench, sterilize the outer surface of the vial by wiping with an alcohol cotton pellet and leave it to dry. Transfer the thawed cell suspension to a 15ml centrifuge tube.

3)Inside the ultra-clean bench, get 5ml EZ-Stem Complete Medium (corresponding to 300ul of cryopreservation solution) and add the medium dropwise by a pipette to the 15ml centrifuge tube which already contains the thawed cell suspension, close the lid, mix by inverting up and down for 3 times, gently and slowly. Then centrifuge at 700rpm for 5 mins at room temp to collect the cells.

4)Inside the ultra-clean bench, carefully remove and discard the supernatant. Resuspend cell pellet with 1ml of EZ-Stem Complete Medium (EZ-Stem Apoptosis inhibitor Medium-containing) and then transfer to a 6-well plate containing 1 ml of EZ-Stem Complete Medium (EZ-Stem Apoptosis inhibitor Medium-containing), label the plate with cell name, date and passage no., incubate the plate in a 37℃, 5%CO2 incubator.

Note: Please thaw the cells into 3 wells of a 6-well plate.

Cell Passaging

1)As long as the cells are 80%-90% confluent, it is ready to passage. Inside the ultra-clean bench, remove and discard the medium from the 6-well plate and briefly rinse the cells with 1ml of 1×PBS for 1-2 times to remove residual medium;

2)Add 0.5ml of EZ-Stem Passaging Medium, gently shake the plate to allow EZ-Stem Passaging Medium completely cover the cells, place the plate into the 37℃ incubator and incubate for 3-5 mins until the majority of the cells have significant retraction (colony pulling away from the matrix layer) under the microscope, remove and discard the EZ-Stem Passaging Medium. Then add the fresh EZ-Stem Complete Medium, gently pipet the cells to cell suspension (do not make it into single cell), and passage the cells at appropriate passage ratio.

3)1:6-1:8 for the first passage, increasing the passaging ratio if the cells are grown to confluence within

two days, or decreasing the passaging ratio if the cells are not grown to confluence in 3-4 days.

Cell cryopreservation

1)Based on the 6-well plate, as long as the cells are 70%-80% confluent (not 100%), it is feasible for cryopreservation. Inside the ultra-clean bench, add 1×PBS to the 6-well plate and briefly rinse the cell 1-2 times, add EZ-Stem Passaging Medium for digestion for 5-10 mins, then gently pipet the cells to the single cell suspension and add the fresh EZ-Stem Complete Medium to terminate digestion. All liquid is transferred to a 15ml or 50 ml centrifuge tube.

2)Centrifuge at 300g for 3 mins at room temp. After centrifugation, remove and discard the supernatant, and add 600 μL EZ-Stem Cryopreservation Medium and 0.15 μL EZ-Stem Apoptosis inhibitor Medium then aliquot into 2 vials (each well can cryopreserve 2 vials).

3)Place the cryovials in a cell freezing container, record the information for the cryopreserved cells, put the container in a -80℃ freezer overnight, then transfer the cryovials to liquid nitrogen on the next day.