Cell Use Instruction - THP-1-CAS9 Cell Line

Product Info

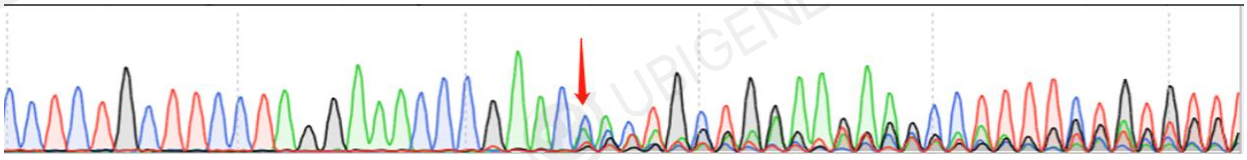
Catalog	YC-D011-Cas9-H		
Cell line	THP-1-CAS9	Morphology	Monocyte,suspension
Fluorescent & resistance	No fluorescence，Hygro	Passage ratio	1：2-1：4
Culture method	90%RPMI-1640+10%FBS Ubigene didn't use P/S. But client could use P/S after cells grow in good condition after thawing.		
Cryopreservation solution	90%FBS+10%DMSO	Antibiotic concentration for maintenance	H=100.0 μg/ml
Special Note	The cell culture density needs to be strictly controlled. The cell viability after thawing is relatively low, it is recommended to use Australia-sourced FBS for thawing or increase the FBS ratio to 20%. When the cells grow normally and can be passaged after 2 passages, the cells can be cultured using the normal culture method.		

Product Validation Data

1) RT-QPCR

Sample Name	Target Name	Cт Mean	ΔCт
THP-1-CAS9	Cas9	18.14743614	4.86366844
THP-1-CAS9	β-actin	13.28376770	
THP-1	Cas9	34.46055222	21.80498600
THP-1	β-actin	12.65556622	

2) Cutting Efficiency Validation



Note: The above figure shows the sequencing peaks of the THP-1-CAS9 stable cell pool which is electroporated by NONO 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

1) Preparation: warm up the complete culture medium in 37°C water bath for 30 mins. Transfer the cryopreserved vial from liquid nitrogen to - 80°C freezer, and leave for several minutes to volatilize residual liquid nitrogen;
2) Inside the ultra-clean bench, pipet 6-7 mL of complete medium into a 15 mL centrifuge tube;
3) Take out the cryopreserved vial from - 80℃ freezer and leave in dry ice temporarily, shake slightly before thawing to remove residual dry ice and liquid nitrogen. 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;
4) 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 cells to the prepared centrifuge tube (step 2) by pipette, close the lid, and centrifuge at 1100 rpm for 4 mins at room temp to collect the cells;
5) Inside the ultra-clean bench, carefully remove and discard the supernatant. Resuspend cell pellet

with 1mL of fresh complete medium and then transfer to a T25 flask (or 6 cm culture dish) containing 4 mL of complete medium, label the flask with cell name, date and passage no., incubate the flask in a 37℃, 5%CO2 incubator.

Note: Please do not thaw the cells directly to a T75 flask or 10 cm culture dish.

Cell Passaging

The cells can be passaged when they have grown to the required density. The passaging of suspension cells can be divided into the following two cases:

a. Half medium replacement: when cells in good condition, with less cell debris and no yellowing of the culture medium, use half medium replacement method for passaging;

1) Inside the ultra-clean bench, gently pipet the cells in the culture flask evenly and take 20 ul of cells for cell counting;

2) According to the cell counting results, aspirate and discard part of the cell suspension, adjust the cell density to $2x10^5$~$4.0x10^5$cells/mL, and culture the cells in different sizes of culture flasks depending on the cell density.

b. Total medium replacement: cells in good condition, with a lot cell debris and the medium has turned yellow, use total medium replacement method for passaging;

1) Transfer culture medium to a 15 mL or 50 mL centrifuge tube in an ultra-clean bench and centrifuge at 1100 rpm for 4 minutes;

2) After centrifugation, remove and discard the supernatant and resuspend the cells with 1 mL of complete medium by pipette, and take 20 ul of cells for cell counting;

3) According to the cell counting results, aspirate and discard part of the cell suspension, adjust the cell density to $2x10^5$~$4.0x10^5$cells/mL, and culture the cells in different sizes of culture flasks depending on the cell density, incubate the flask in a 37℃, 5%CO2 incubator.

Table 1. List of different volumes of medium for suspension cells in different culture plates/flasks

Size of culture plates/flasks	Volume of culture medium
6-well plate	3 mL
T25	5mL-8mL
T75	12mL-28mL
T175	30mL-50mL

Note: In order to maintain the stable expression of Cas9 gene, it is recommended to add antibiotics for culture when cell viability >70% (see the concentration for maintenance above).

Cell cryopreservation

1) Same as procedures of cell passaging, transfer cells from culture flasks to 50 mL centrifuge tubes in an ultra-clean bench and centrifuge at 1100 rpm for 4 minutes at room temp;

2) After centrifugation, remove and discard the supernatant, and resuspend the cells with 1-2 mL of 4℃ pre-cooled cryopreservation medium (use the one you usually use in lab, or any commercial cryopreservation solutions are fine), mix well by pipetting and take 20 μL for cell counting, then add cryopreservation medium to adjust to the required density ($5×10^6$-$1x10^7$cells/mL);

3) Aliquot the cell suspension to cryovials as 1 mL/tube, close the lid tightly, and the cryovials should

be labeled with the cell name, source, cell passage number, and date of cryopreservation in advance;

4) Place the cryovials in 4℃ pre-cooled Freezing Container, then put the container in -80℃ freezers within 15 mins after cell cryopreservation;

5) Stay overnight, transfer the cryovials to liquid nitrogen for long-term storage.