

## Cell Use Instruction - RAW 264.7-CAS9 Cell Line

### Product Info

|  |  |               |                               |
|--|--|---------------|-------------------------------|
| Catalog                                  | YC-C020-Cas9-H   |               |                               |
| Cell line                                | RAW 264.7-CAS9   | Morphology    | monocyte/macrophage, adherent |
| Fluorescent & resistance                 | No fluorescence, Hygro   | Passage ratio | 1:2-1:3                       |
| Culture method                           | 90%DMEM+10%FBS<br>Ubigene didn't use P/S. But client could use P/S after cells grow in good condition after thawing.                                     |               |                               |
| Cryopreservation solution                | 50%DMEM+40% FBS+10%DMSO  |               |                               |
| Antibiotic concentration for maintenance | H=350.0μg/mL<br><b>Note: To maintain stable expression of the cas9 gene, maintain cells under antibiotic selection during optimal growth conditions.</b> |               |                               |
| Special Note                             | Cells cannot be digested with trypsin. Cells need to be digested by gently pipetting into single cells.  |               |                               |

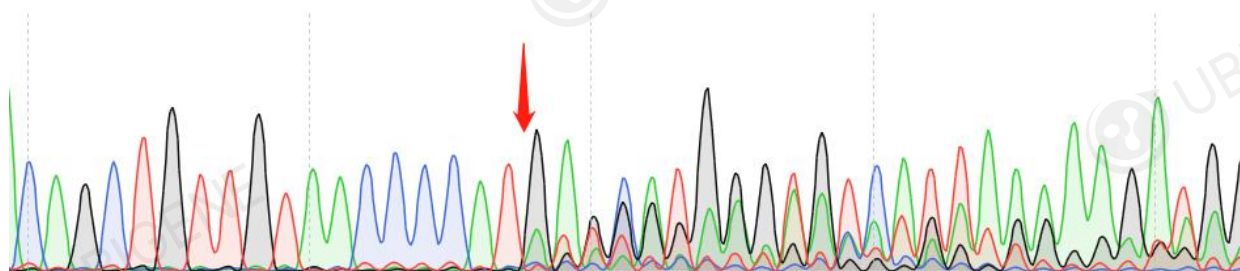
### Product Validation Data

#### 1) RT-QPCR

| Sample Name    | Target Name | Ct Mean     | ΔCt        |
|----------------|-------------|-------------|------------|
| RAW 264.7-CAS9 | Cas9        | 20.70931435 | 8.58496571 |
| RAW 264.7-CAS9 | β-actin     | 12.12434864 |            |

|           |                |             |             |
|-----------|----------------|-------------|-------------|
| RAW 264.7 | Cas9           | 34.13895035 | 21.32848454 |
| RAW 264.7 | $\beta$ -actin | 12.81046581 |             |

## 2) Cutting Efficiency Validation



Note: The above figure shows the sequencing peaks of the RAW 264.7-CAS9 stable cell pool which is electroporated by Ppt1 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 take 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°C 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°C, 5%CO<sub>2</sub> incubator.

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

## Cell Passaging

- 1) Cells need to be passaged daily. During cell passaging, inside the ultra-clean bench, gently pipet the cells into single cells (be sure to observe all cells not in clumps, and into single cells under the microscope);
- 2) Transfer the cell suspension into a 15 mL or 50 mL centrifuge tube, cells from same batch can be collected and put together, then put the cap and label;
- 3) Centrifuge at 1100 rpm for 4 mins at room temp. After centrifugation, remove and discard the supernatant and resuspend the cells with 2 mL of complete medium;
- 4) Cells need to be passaged at appropriate passage ratio, 1:2 for the first passage.

## Cell cryopreservation

- 1) Same as procedures of cell passaging, inside the ultra-clean bench, digest the cells to a single-cell suspension, and terminate digestion by adding complete medium. All liquid is transferred to a 50

mL centrifuge tube;

- 2) Mix well by pipetting and take 20  $\mu$ L for cell counting;
- 3) Centrifuge at 1100 rpm for 4 mins at room temp. After centrifugation, remove and discard the supernatant, and resuspend the cells with 1-2 mL of 4°C pre-cooled cryopreservation medium (use the one you usually use in lab, or any commercial cryopreservation solutions are fine), then add cryopreservation medium to adjust to the required density ( $1 \times 10^6$ - $1 \times 10^7$  cells/mL);
- 4) 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;
- 5) Place the cryovials in 4°C pre-cooled Freezing Container, then put the container in -80°C freezers within 15 mins after cell cryopreservation;
- 6) Stay overnight, transfer the cryovials to liquid nitrogen for long-term storage.