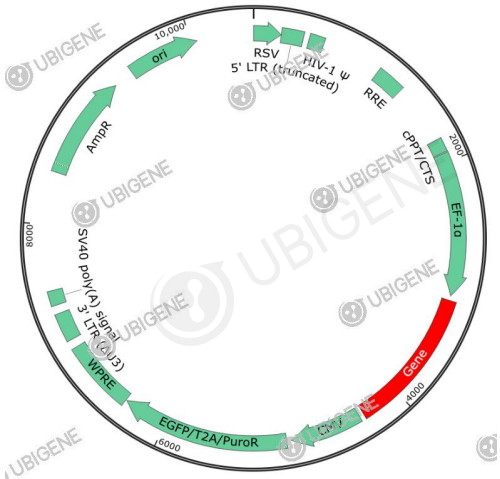
Cell Use Instruction - Gpx4 Stable Expression Cell Line (RAW 264.7)

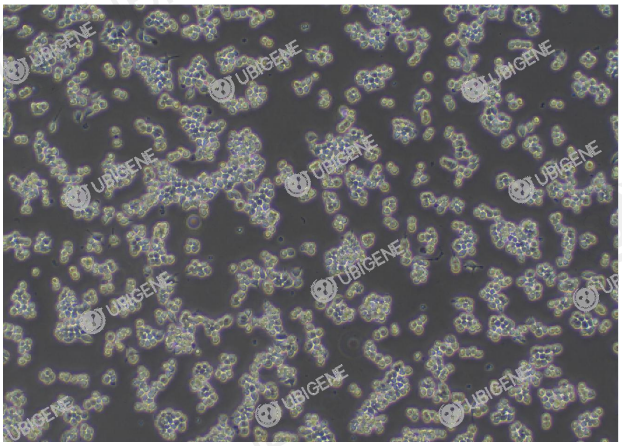
Product Info

Catalog	YOE-L029		
Cell line	Gpx4 Stable Expression Cell Line (RAW 264.7)	Morphology	monocyte/macrophage,adherent
Gene Name	Gpx4	Passage ratio	1:2-1:3
Culture method	90%DMEM+10%FBS 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	Puro=3μg/mL Note: To maintain stable expression of the gene, maintain cells under antibiotic selection during optimal growth conditions.		
Special Note	Cells cannot be digested with trypsin. Cells need to be digested by gently pipetting into single cells.		

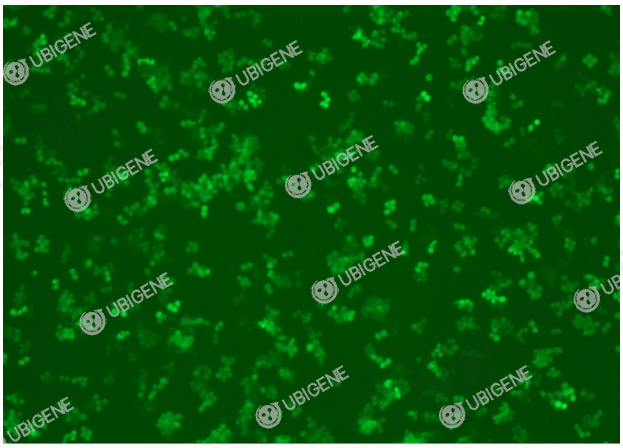
Plasmid Backbone Map



Cell image



Gpx4 Stable Expression Cell Line (RAW 264.7)-100X



Gpx4 Stable Expression Cell Line (RAW 264.7)-100X-300ms

RT-QPCR result

(1) Primers

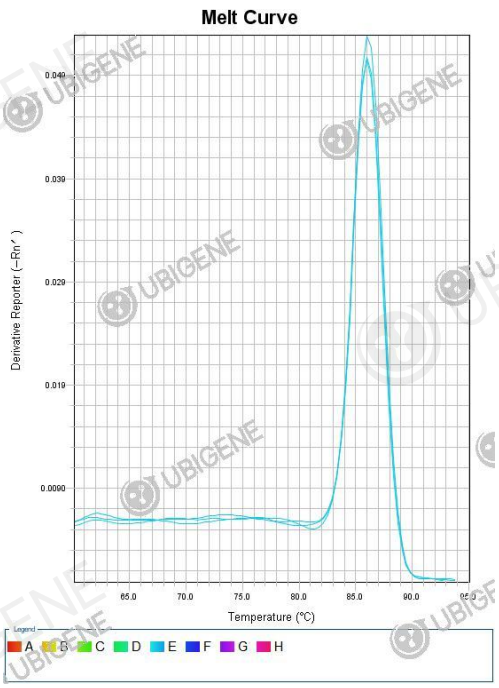
Name	Primer	Sequence	Size
mGpx4-1	Forward	5’-GCCAAAGTCCTAGGAAACGC -3’	111bp
	Reverse	5’-CCGGGTTGAAAGGTTCAGGA-3’	
Q-mGAPDH	Forward	5’-GTCAAGGCCGAGAATGGGAA-3’	237bp
	Reverse	5’-CTCGTGGTTCACACCCATCA-3’	

(2) CT value

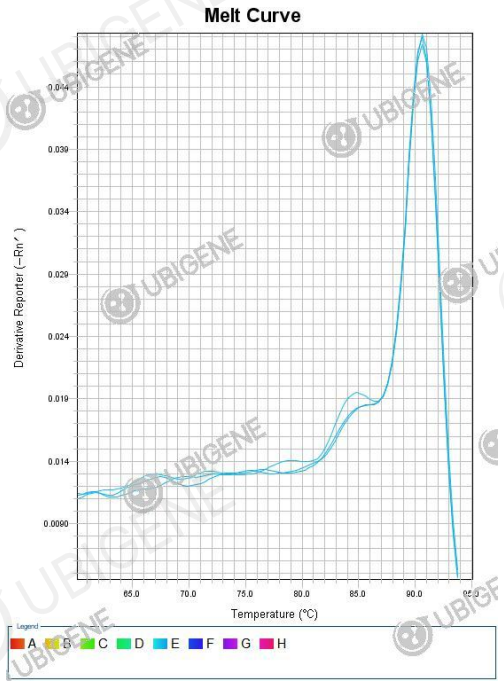
No.	Sample	mGpx4-1 Cт Mean	Q-mGAPDH Cт Mean	ΔCт Mean	ΔΔCт	Expression Rate
1	Gpx4 Stable Expression Cell Line (RAW 264.7)	23.977	13.356	10.621	-6.410	85.036
2	RAW264.7_Ctrl(Puro)	30.749	13.718	17.031	0.000	1.000

(3) Melt curve

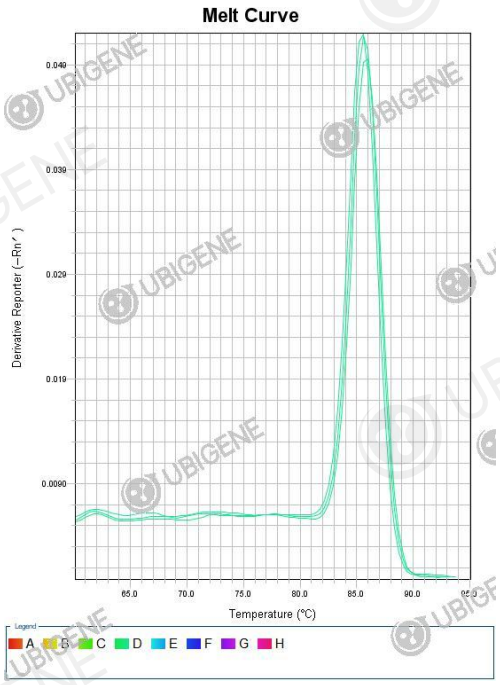
Gpx4 Stable Expression Cell Line (RAW 264.7)+mGpx4-1 melt curve



Gpx4 Stable Expression Cell Line (RAW 264.7)+Q-mGAPDH melt curve

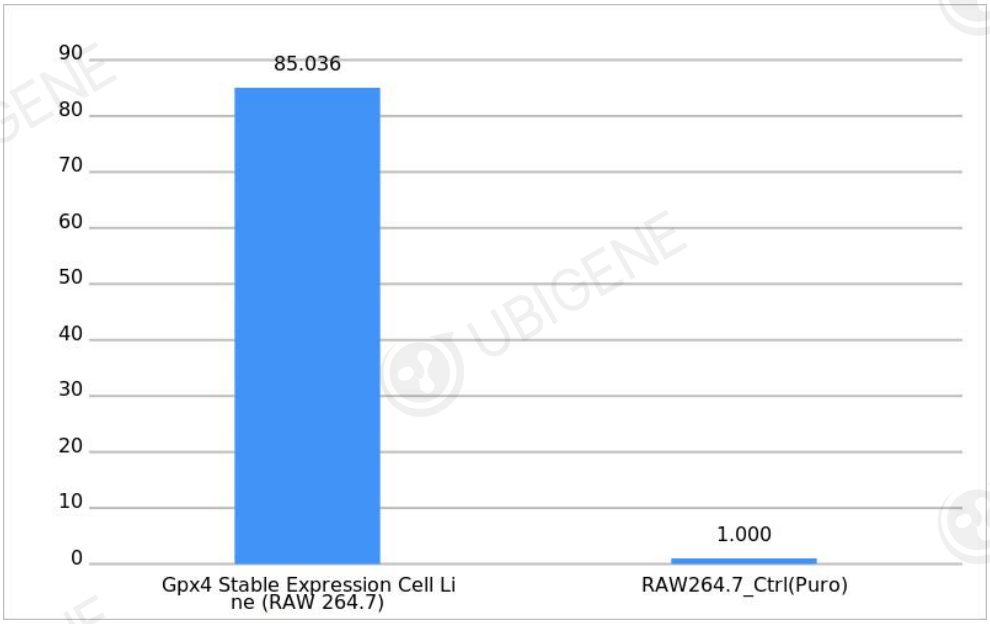


RAW264.7_Ctrl(Puro)+mGpx4-1 melt curve



RAW264.7_Ctrl(Puro)+Q-mGAPDH melt curve

(4) Gene Expression



(5) Conclusion

Compared with RAW264.7_Ctrl(Puro), the expression rate of Gpx4 Stable Expression Cell

Line (RAW 264.7) cell line was 8503.6%, The generation of stable Gpx4-overexpression cell line was successful.

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

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 μL 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℃ 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 ($1x10^6$-$1x10^7$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℃ freezers within 15 mins after cell cryopreservation;

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