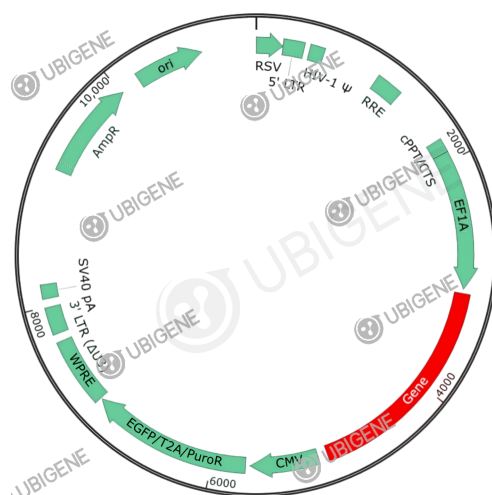


Cell Use Instruction - LAG3 Stable Expression Cell Line (Jurkat, Clone E6-1)

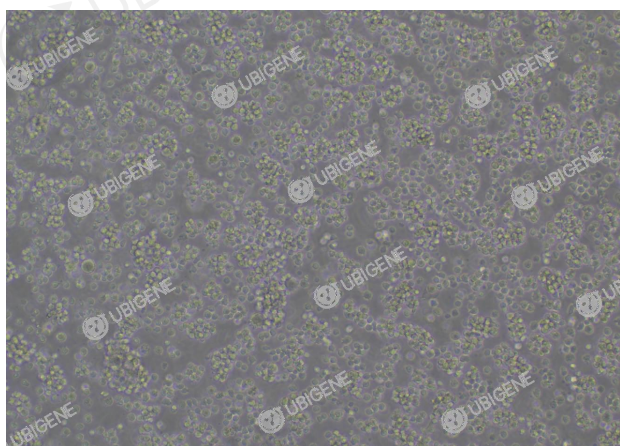
Product Info

| | | | |
|--|--|---------------|----------------------------|
| Catalog | YOE-L080 | | |
| Cell line | LAG3 Stable Expression Cell Line (Jurkat, Clone E6-1) | Morphology | Lymphocyte-like,suspension |
| Gene Name | LAG3 | Passage ratio | 1:3-1:5 |
| 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 | 50%RPMI-1640+40%FBS+10%DMSO | | |
| Antibiotic concentration for maintenance | Puro=2μg/mL Note: To maintain stable expression of the gene, maintain cells under antibiotic selection during optimal growth conditions. | | |
| Special Note | The cell culture density needs to be strictly controlled. | | |

Plasmid Backbone Map



Cell image



LAG3 Stable Expression Cell Line (Jurkat, Clone E6-1)-

100X



LAG3 Stable Expression Cell Line (Jurkat, Clone E6-1)-

100X-300ms

RT-QPCR result

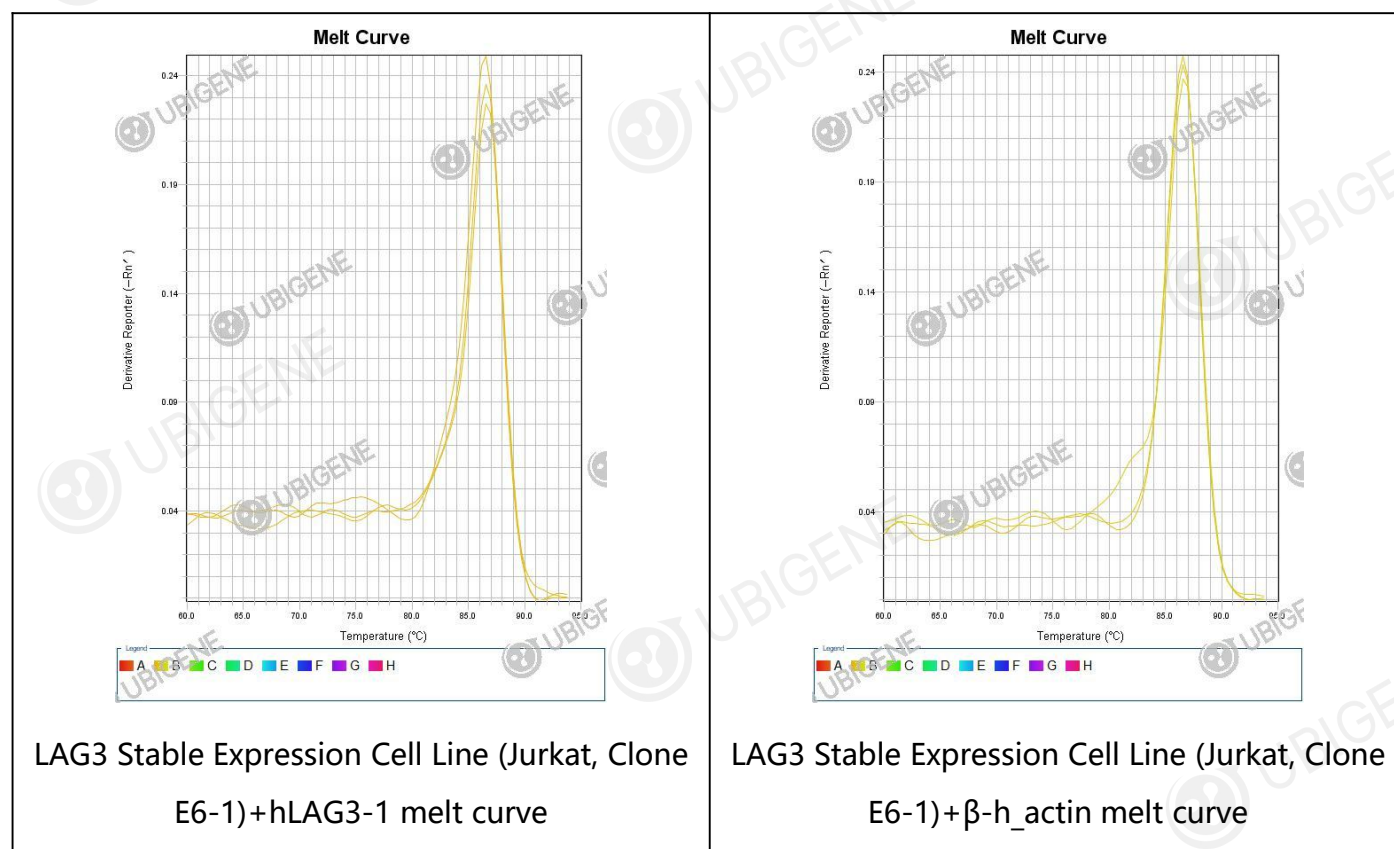
(1) Primers

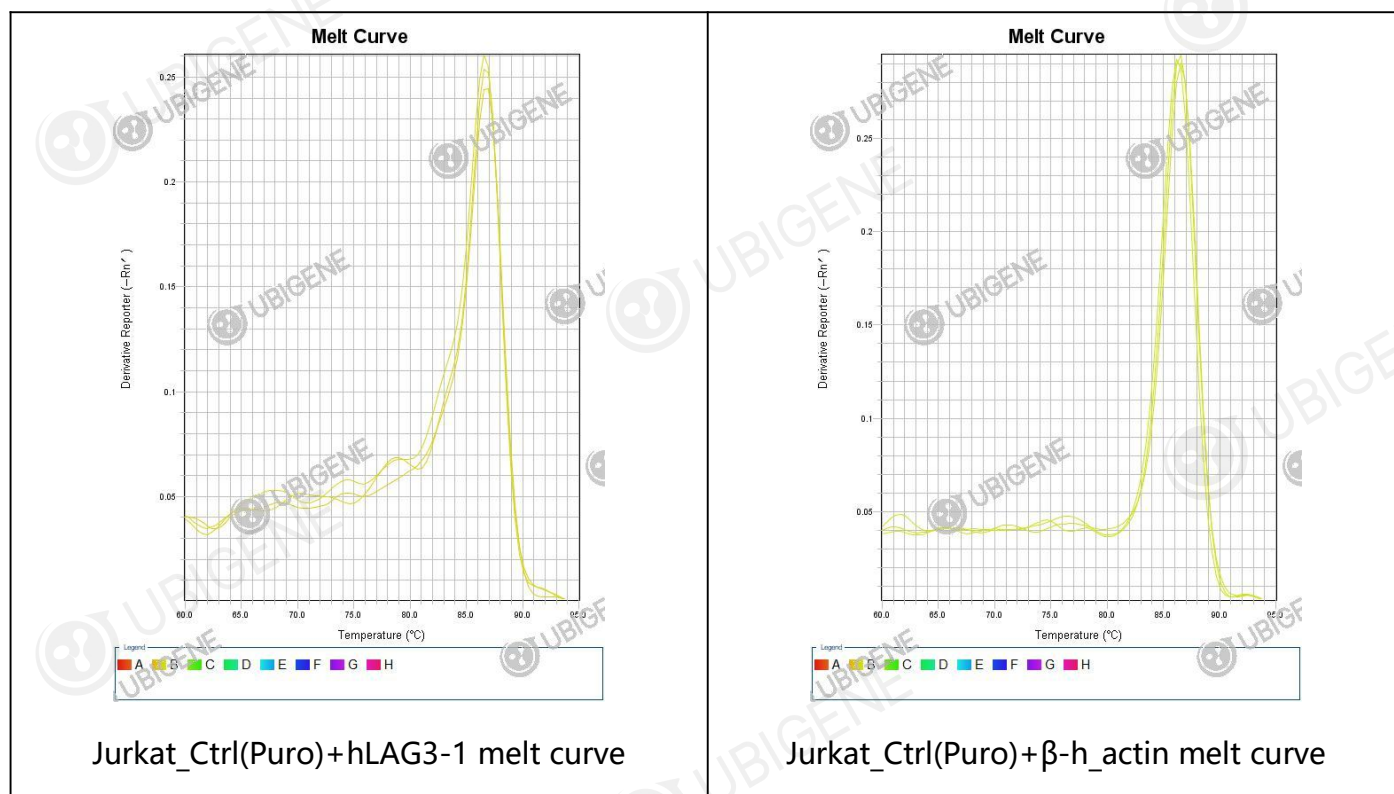
| Name | Primer | Sequence | Size |
|-----------|---------|-----------------------------|--------|
| hLAG3-1 | Forward | 5'-GGCTGCATCCTCACCTACAG -3' | 173 bp |
| | Reverse | 5'-TTGGCAGTGAGGAAAGACCG-3' | |
| β-h_actin | Forward | 5'-CGAGCACGGCATCGTCAC-3' | 199bp |
| | Reverse | 5'-CTGGATAGCAACGTACATGGC-3' | |

(2) CT value

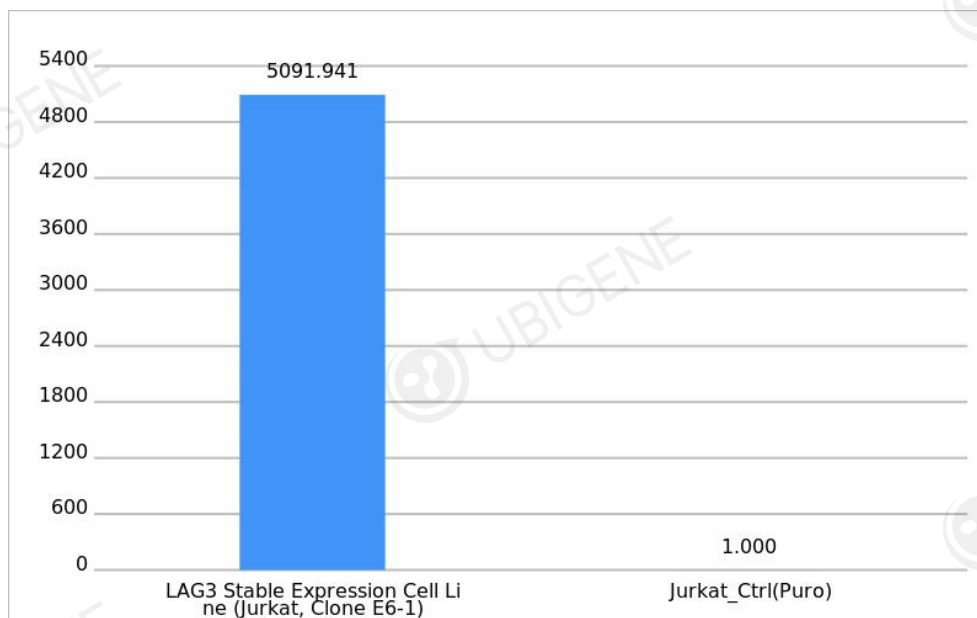
| No. | Sample | hLAG3-1 C _T Mean | β-h_actin C _T Mean | ΔC _T Mean | ΔΔC _T | Expression Rate |
|-----|---|--------------------------------|----------------------------------|-------------------------|------------------|--------------------|
| 1 | LAG3 Stable Expression Cell Line (Jurkat, Clone E6-1) | 17.849 | 14.674 | 3.175 | - 12.314 | 5091.941 |
| 2 | Jurkat_Ctrl(Puro) | 30.042 | 14.553 | 15.489 | 0.000 | 1.000 |

(3) Melt curve





(4) Gene Expression



(5) Conclusion

Compared with Jurkat_Ctrl(Puro), the expression rate of LAG3 Stable Expression Cell

Line (Jurkat, Clone E6-1) cell line was 509194.1%, The generation of stable LAG3-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 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%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 $2 \times 10^5 \sim 4.0 \times 10^5$ 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 μ L 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 $2 \times 10^5 \sim 4.0 \times 10^5$ cells/mL, and culture the cells in different sizes of culture flasks depending on the cell density, incubate the flask in a 37°C, 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 |

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°C 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 μ L for cell counting, then add cryopreservation medium to adjust to the required density (5×10^6 - 1×10^7 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°C pre-cooled Freezing Container, then put the container in -80°C freezers within 15 mins after cell cryopreservation;
- 5) Stay overnight, transfer the cryovials to liquid nitrogen for long-term storage.