Certificate of Analysis

Cat. No.: RD00741

GenScript USA, Inc.
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Contents

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Certificate of Analysis

Project Information

Order Number: RD00741

Host Cell: CHO-K1

Target Gene: LAG3

Product Information

	Cell Name	Cell Culture Medium	Freeze Medium
Product	CHO-K1/LAG3	F-12K with 10% FBS, 8 μg/mL puromycin	Complete growth medium with 5% (V/V) DMSO

Materials

Materials	Company	Cat. No.
F-12K	Gibco	21127-022
Puromycin	Gibco	A1113803
FBS	BIOIND	04-001-1A
0.25% Trypsin	Gibco	25200-072
DMSO	SIGMA	D2650

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QC Results

Validation by Flow Cytometry

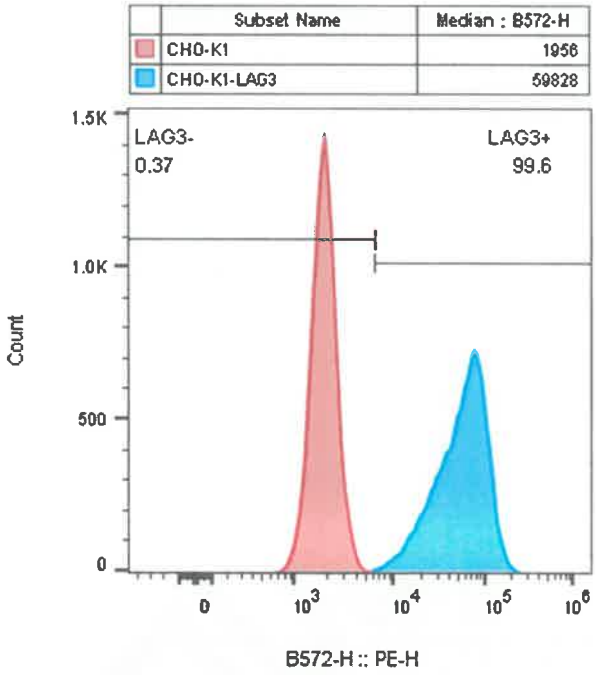

Figure1. Flow cytometric analysis of LAG3 in CHO-K1/LAG3 with anti-Human LAG-3.

Mycoplasma Testing:

Cell	Value*	Result**
CHO-K1/LAG3	0.60	Passed

**The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit. MycoAlert PLUS is able to detect almost all common Mollicutes contaminations with the exception of Ureaplasma that utilizes a different metabolic pathway and lacks the enzyme the kit detects. As Ureaplasma is not included on the list of the organisms required by the FDA/USP, it is considered unlikely to ever see this contamination in a cell culture.*

***The test has been designed to give ratios of less than 1 with uninfected samples and routinely yield ratios greater than 1 for samples infected with mycoplasma.*

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Appendix

Target Gene Information:

Human LAG3: NM_002286.5

Instruction for Maintaining Stable Cell Line

Cell Recovery

The cells were maintained in F-12K supplemented with 10% FBS, and 8 μg/mL puromycin.

The standard operating procedure for cell recovery is briefly described here:

1) Prewarm a water bath to 37°C.
2) Remove the cryovial from the liquid nitrogen tank and thaw by gentle agitation in a 37°C water bath until ice crystals are melted, usually within 2-3 minutes.
3) Remove the vial from the water bath and decontaminate it by spraying with 75% ethanol.
4) Unscrew the vial and transfer the cells to a 15-mL sterile conical centrifuge tube containing 9 mL complete growth medium.
5) After centrifugation at 800 rpm for 4 minutes, discard the supernatant and resuspend the cell pellet in 2 mL of complete growth medium. Pipette gently to loosen the pellet and break apart clumps.
6) Transfer the cell suspension to the culture vessel with antibiotic free medium and mix thoroughly. Incubate cultures at 37°C, 5% CO_2.
7) Aspirate the medium and replace with fresh culture medium the next day.

Cell Maintenance and Subculturing

The steps described below is specifically designed for T-75 flask.

TIPS Proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1) Prewarm the complete growth medium to 37°C in a water bath.
2) Remove culture medium in the flask.
3) Briefly rinse the cell layer with Ca^{2+}/Mg^{2+} free DPBS to remove the residual serum.
4) Add 1.0-2.0 mL of Trypsin-EDTA solution to the flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 1 to 3 minutes).

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TIPS To avoid clumps, do not agitate the cells by hitting or shaking the flask during detachment.

5) Add 8.0 mL of complete growth medium and aspirate cells by gently pipetting.

6) Centrifuge at 800 rpm for 4 minutes, discard the supernatant and resuspend the cell pellet in 5.0 mL of complete growth medium.

7) Add appropriate aliquots of the cell suspension to new culture flasks. Incubate cultures at 37°C.

Subcultivation Ratio: 1:6 to 1:10

Medium Renewal: Every 2 to 3 days

Cell Cryopreservation

1) Prepare a freeze medium consisting of complete growth medium supplemented with 5% DMSO.

2) Harvest cells by gentle centrifugation at 800 rpm for 4 minutes and resuspend them in the freeze medium at a concentration of 1×10^6 viable cells/mL. Continue to culture the cells until the viability of the recovered cells is confirmed.

3) Label the cryovials with the name of the cell line, then add 1 mL of the cell suspension to each of the vials and seal the cap.

4) Place the vials into a pre-cooled (4°C) controlled-rate freeze chamber and place the chamber in a -80°C freezer for at least 24 hours.

5) Quickly transfer the vials to a liquid nitrogen tank.

6) After 24 hours in liquid nitrogen, take one vial and recover the cells to determine the cell viability.

Packing List

Cell (Shipping Condition: Liquid nitrogen, Store at -196°C)

Name: CHO-K1/LAG3

Quantity: 1×10^6 cells/vial

Lot No.: RD00741/PD04ED012

Number of vial: 2 vials

Store at: -196°C

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