293T Cyno LAG3 Cell Line

Cat. No: KC-0208 Version 17123101

I. Cell Line Information

Catalog number	KC-0208
Cell line name:	293T cyno LAG3 Cell Line
Gene ID/Accession #:	XM_005569954.2
Host cell line	293T
Cell type:	Human embryonic kidney
Description:	HEK293T cell line stable expressing exogenous cynomolgus LAG3 gene
Quantity:	One vial of frozen cells ($5X10^6$ per vial)
Stability:	Stable in culture over a minimum of 10 passages
Application:	Drug screening and biological assays
Freeze medium:	70% DMEM + 20% FBS + 10% DMSO
Propagation medium:	DMEM + 10% FBS + 0.5ug/ml Puromycin
Selection marker:	Puromycin
Morphology:	Fibroblastoid cells growing as monolayer
Subculture:	Split saturated culture 1:4~1:5 every 2~3 days; seed out at about 1-3 x 10^5 cells/ml
Incubation:	37 ℃ with 5% CO_2
Doubling time:	Approximately 30 hours
Mycoplasma status:	Negative
Biosafety level:	1
Storage:	Liquid nitrogen immediately upon receiving

II. Background

Lymphocyte-activation gene 3(LAG3), also known as CD223, is a member of immunoglobulin superfamily, expressed on activated T cells, NK cells, B cells and DC cells. LAG3 can bind to MHC II molecules and induce maturation of DC cells, and cytokine secretion of cytotoxic T cells and NK cells. LAG3 is a promising drug target of cancer therapy and autoimmune disease, its therapeutic antibodies including BMS-986016 and GSK2831781 had

already been used in the clinical trial.

III. Cell Line Generation

293T cyno LAG3 cell line was generated using lentiviral vector expressing cynomolgus LAG3 sequence.

IV. Characterization using FACS

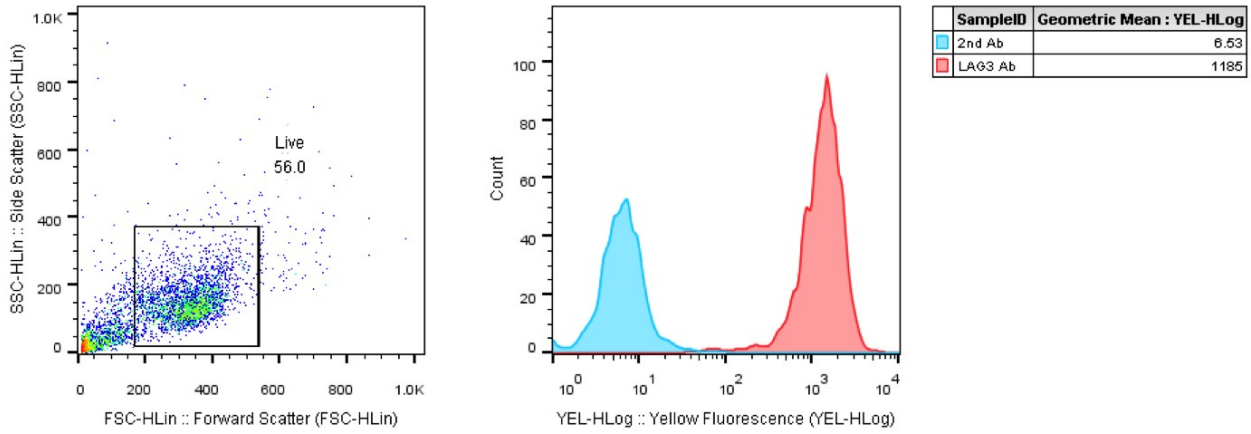

Figure: Characterization of LAG3 overexpressing in 293T stable clones using LAG3.

V. Application

Hybridoma or Binders of ligand screening with FACS.

VI. Cell Resuscitation

1. Prewarm culture medium (DMEM supplemented with 10% FBS and 0.5ug/ml puromycin) in a 37℃ water bath.
2. Thaw the frozen vial in a 37℃ water bath for 1-2 minutes.
3. Transfer the vial into biosafety cabinet, and wipe the surface with 70% ethanol.
4. Unscrew the top of the vial and transfer the cell suspension gently into a sterile centrifuge tube containing 9.0 mL complete culture medium.
5. Spin at ~ 125 x g for 5~7 minutes at room temperature, and discard the supernatant without disturbing the pellet.
6. Resuspend cell pellet with the appropriate volume of complete medium and transfer the cell suspension into a T25 culture flask.
7. Incubate the flask at 37℃, 5% CO_2 incubator.
8. Split saturated culture 1:4 ~ 1:5 every 2~3 days; seed out at about 1-3 x 10^5 cells/ml.

VII. Cell Freezing

1. Prepare the freezing medium (70% DMEM + 20% FBS + 10% DMSO) fresh immediately before use.
2. Keep the freezing medium on ice and label cryovials.
3. Transfer cells to a sterile, conical centrifuge tube, and count the cells.
4. Centrifuge the cells at 250 x g for 5 minutes at room temperature and carefully aspirate off the medium.
5. Resuspend the cells at a density of at least 3 x10^6 cells/ml in chilled freezing medium.
6. Aliquot 1 ml of the cell suspension into each cryovial.
7. Freeze cells in the CoolCell freezing container overnight in a −80°C freezer.
8. Transfer vials to liquid nitrogen for long-term storage.

VIII. References

1. Anderson, Ana C, Nicole Joller, and Vijay K Kuchroo. 2016. “Lag-3, Tim-3, and TIGIT: Co-Inhibitory Receptors with Specialized Functions in Immune Regulation.” Immunity 44 (5). Elsevier Inc.: 989–1004.
2. Huard, B, R Mastrangeli, P Prigent, D Bruniquel, S Donini, N El-Tayar, B Maigret, M Dréano, and F Triebel. 1997. “Characterization of the Major Histocompatibility Complex Class II Binding Site on LAG-3 Protein..” Proceedings of the National Academy of Sciences 94 (11): 5744–49.

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