CHO-K1 human CD154(CD40L) Cell Line

Cat. No: KC-1316 Version 19062801

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I. Cell Line Information

Catalog number	KC-1316
Cell line name:	CHO-K1 human CD154(CD40L) Cell Line
Gene ID/Accession #:	NM_000074.2
Host cell line	CHO-K1
Cell type:	Chinese hamster ovary cell line
Description:	CHO-K1 cell line stable expressing exogenous human CD154 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% F12K + 20% FBS + 10% DMSO
Propagation medium:	F12K + 10% FBS + 5ug/ml Puromycin
Selection marker:	Puromycin
Morphology:	Epithelial
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

CD154, also named as CD40L ligand, is protein of TNF superfamily, mainly expressed on activated T cell, functioned as CD40 ligand, plays critical in immune response, such as B cell maturation, macrophage activation.

III. Cell Line Generation

CHO-K1 human CD154 cell line was generated using lentiviral vector expressing human CD154 sequence.

IV. Characterization using FACS

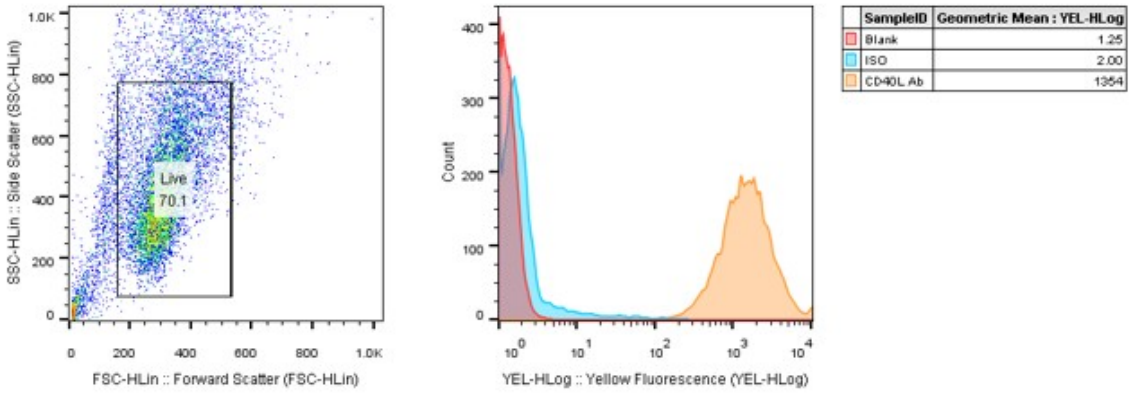

Figure: Characterization of CD154 overexpressing in CHO-K1 stable clones using FACS.

V. Application

Hybridoma or Binders of ligand screening with FACS.

VI. Cell Resuscitation

1. Prewarm culture medium (F12K 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% F12K + 20% FBS + 10% DMSO) fresh immediately before use.
2. Keep the freezing medium on ice and label cryovials.
3. Harvest cells to a sterile, conical centrifuge tube during the logarithmic growth period 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. Lederman S, Yellin MJ, Krichevsky A, Belko J, Lee JJ, Chess L (April 1992). "Identification of a novel surface protein on activated CD4+ T cells that induces contact-dependent B cell differentiation (help)". The Journal of Experimental Medicine. 175 (4): 1091–101.
2. Lederman S, Yellin MJ, Inghirami G, Lee JJ, Knowles DM, Chess L (December 1992). "Molecular interactions mediating T-B lymphocyte collaboration in human lymphoid follicles. Roles of T cell-B-cell-activating molecule (5c8 antigen) and CD40 in contact-dependent help". Journal of Immunology. 149 (12): 3817–26.
3. Lederman S, Yellin MJ, Cleary AM, Pernis A, Inghirami G, Cohn LE, Covey LR, Lee JJ, Rothman P, Chess L (March 1994). "T-BAM/CD40-L on helper T lymphocytes augments lymphokine-induced B cell Ig isotype switch recombination and rescues B cells from programmed cell death". Journal of Immunology. 152 (5): 2163–71.

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