

# 293T human CD40 Cell Line

Cat. No: KC-0222

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## I. Cell Line Information

<b>Catalog number</b>	KC-0222
<b>Cell line name:</b>	293T human CD40 Cell Line
<b>Gene ID/Accession #:</b>	NM_001250.5
<b>Host cell line</b>	293T
<b>Cell type:</b>	Human embryonic kidney
<b>Description:</b>	HEK293T cell line stable expressing exogenous human CD40 gene
<b>Quantity:</b>	One vial of frozen cells (5X10 <sup>6</sup> per vial)
<b>Stability:</b>	Stable in culture over a minimum of 10 passages
<b>Application:</b>	Drug screening and biological assays
<b>Freeze medium:</b>	70% DMEM + 20% FBS + 10% DMSO
<b>Propagation medium:</b>	DMEM + 10% FBS + 0.5ug/ml Puromycin
<b>Selection marker:</b>	Puromycin
<b>Morphology:</b>	Fibroblastoid cells growing as monolayer
<b>Subculture:</b>	Split saturated culture 1:4~1:5 every 2~3 days; seed out at about 1-3 x 10 <sup>5</sup> cells/ml
<b>Incubation:</b>	37 °C with 5% CO <sub>2</sub>
<b>Doubling time:</b>	Approximately 30 hours
<b>Mycoplasma status:</b>	Negative
<b>Biosafety level:</b>	1
<b>Storage:</b>	Liquid nitrogen immediately upon receiving

## II. Background

CD40, also named as TNFRSF5, is a transmembrane protein of the TNF receptor superfamily, expressed on B cells, DC cells, macrophage, monocytes and plates, CD40 functioned as a costimulatory molecule and mediated broad immune and inflammatory response.

### III. Cell Line Generation

293T human CD40 cell line was generated using lentiviral vector expressing human CD40 sequence.

### IV. Characterization using FACS

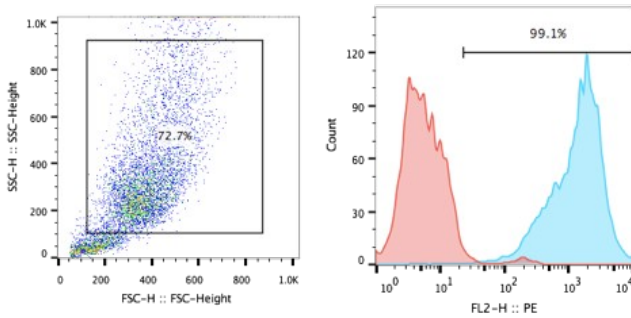


Figure: Characterization of CD40 overexpressing in 293T stable clones using FACS.

### 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°C water bath.
2. Thaw the frozen vial in a 37°C 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°C, 5% CO<sub>2</sub> incubator.
8. Split saturated culture 1:4 ~ 1:5 every 2~3 days; seed out at about 1-3 x 10<sup>5</sup> 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. 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  $3 \times 10^6$  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  $-80^{\circ}\text{C}$  freezer.
8. Transfer vials to liquid nitrogen for long-term storage.

## VIII. References

1. Kawabe, Tsutomu; Naka, Tetsuji; Yoshida, Kanji; Tanaka, Takashi; Fujiwara, Hiroshi; Suematsu, Sachiko; Yoshida, Nobuaki; Kishimoto, Tadamitsu; Kikutani, Hitoshi (1994). "The immune responses in CD40-deficient mice: Impaired immunoglobulin class switching and germinal center formation". *Immunity*. 1 (3): 167–178.
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3. Carling J, Altaher HM, Clark S, Chen X, Latimer SL, Jenner T, Buckle AM, Heath AW (May 2011). "CD154-CD40 interactions in the control of murine B cell hematopoiesis". *Journal of Leukocyte Biology*. 89 (5): 697–706.

**Kyinno Biotechnology Co., Ltd.**

Yizhuang Biomedical Park, No. 88, Beijing, China

Tel: +86-10-58222702

E-mail: [bd@kyinno.com](mailto:bd@kyinno.com)Web: [www.kyinno.com](http://www.kyinno.com)**For Research Use Only**