HT1080 human CD40 Cell Line

Cat. No: KC-0142 Version 17103102

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I. Cell Line Information

Catalog number	KC-0142
Cell line name:	HT1080 human CD40 cell line
Gene ID/Accession #:	NM_001250.5
Host cell line	HT1080
Cell type:	Human fibrosarcoma cells
Description:	HT1080 cell line stable expressing exogenous human CD40 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% RMPI1640 + 20% FBS + 10% DMSO
Propagation medium:	RMPI1640 + 10% FBS + 0.5ug/ml Puromycin
Selection marker:	Puromycin
Morphology:	epithelial 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 °C with 5% CO_2
Doubling time:	Approximately 48 hours
Mycoplasma status:	Negative
Biosafety level:	1
Storage:	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

HT1080 human CD40 cell line was generated using lentiviral vector expressing human CD40 sequence.

IV. Characterization using FACS

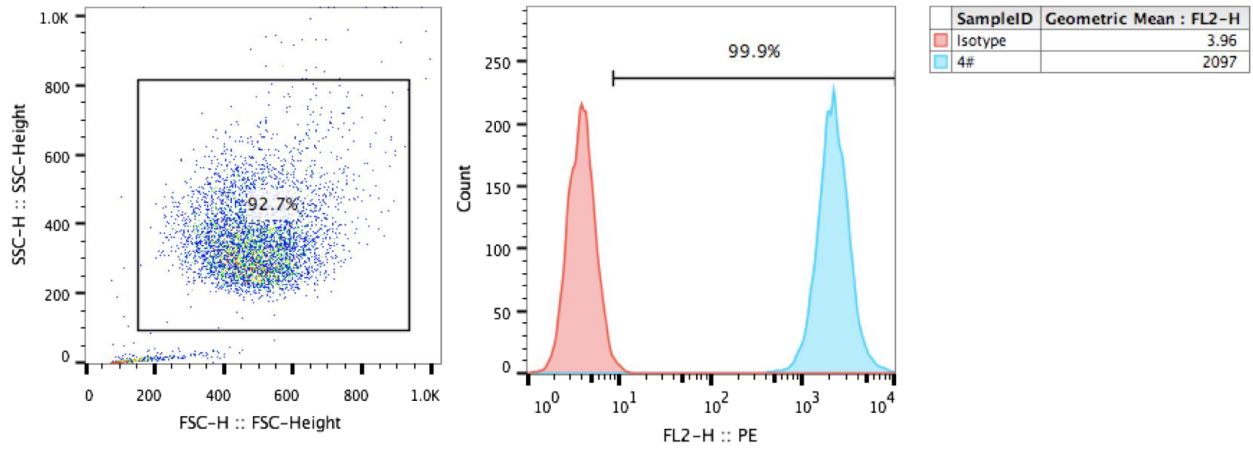
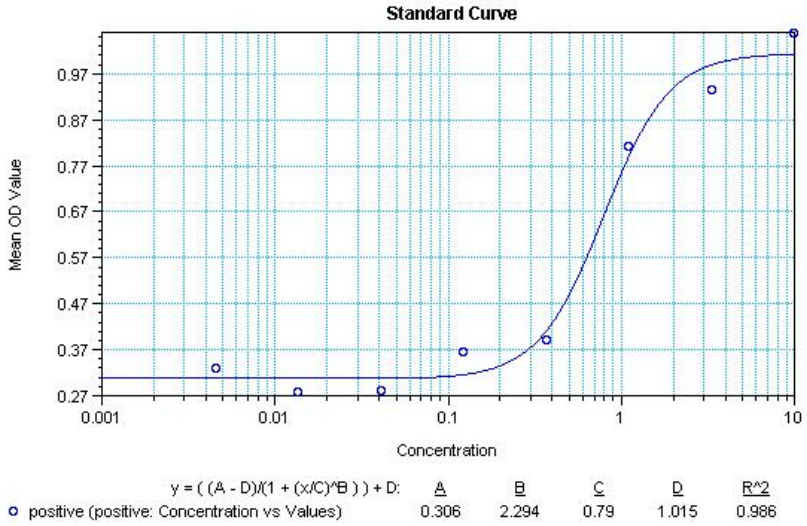


Figure: Characterization of CD40 overexpressing in HT1080 stable clones using FACS.

V. Application

1. Hybridoma or Binders of ligand screening with FACS.
2. Functional assay for CD40 agonist or antagonist.



VI. Cell Resuscitation

1. Prewarm culture medium (RPMI1640 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_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% RMPI1640 + 20% FBS + 10% DMSO) fresh immediately before use.
2. Keep the freezing medium on ice and label cryovials.
3. Harvest the cells during the logarithmic growth period, and 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. 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. Carlring 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.

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