293T human CD28 Cell Line

Cat. No: KC-0211 Version 17103101

Ⅰ. Cell Line Information

Catalog number	KC-0211
Cell line name:	293T human CD28 Cell Line
Gene ID/Accession #:	NM_006139.4
Host cell line	293T
Cell type:	Human embryonic kidney
Description:	HEK293T cell line stable expressing exogenous human CD28 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 °C with 5% CO_2
Doubling time:	Approximately 30 hours
Mycoplasma status:	Negative
Biosafety level:	1
Storage:	Liquid nitrogen immediately upon receiving

Ⅱ. Background

CD28 is a membrane protein of B7 receptor family and mainly expressed on T cells. CD28 is the receptor for CD80 (B7.1) and CD86 (B7.2) proteins, and provide co-stimulatory signals for T cell activation and survival.

Ⅲ. Cell Line Generation

293T human CD28 cell line was generated using lentiviral vector expressing human CD28 sequence.

IV. Characterization using FACS

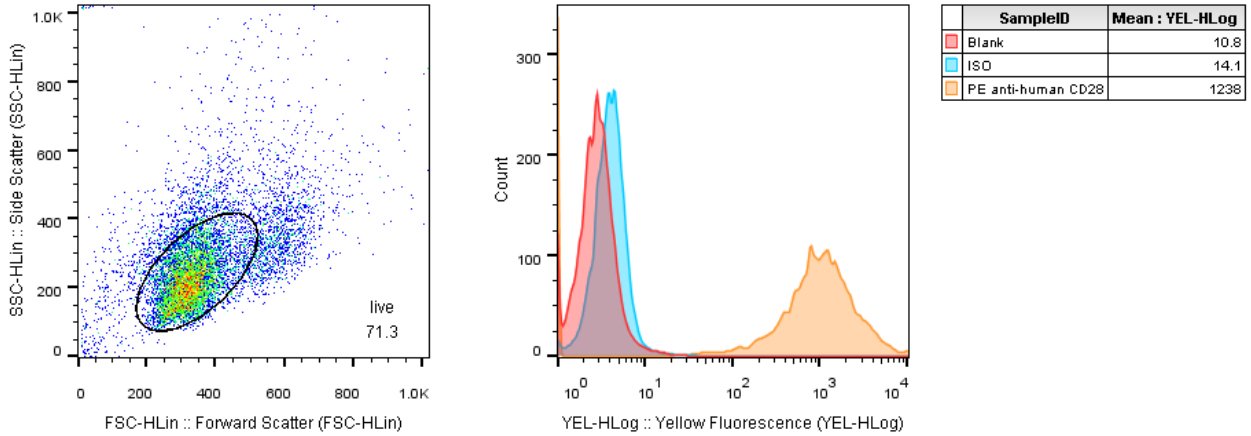

Figure: Characterization of CD28 overexpressing in 293T stable clones using FACS.

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_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. 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. Linsley PS, Ledbetter JA (1993). "The role of the CD28 receptor during T cell responses to antigen". Annu. Rev. Immunol. 11: 191–212.
2. Lenschow DJ, Walunas TL, Bluestone JA (1996). "CD28/B7 system of T cell costimulation". Annu. Rev. Immunol. 14: 233–58.

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