293T OS8 Cell Line

Cat. No: KC-1113 Version 18092002

I. Cell Line Information

Catalog number	KC-0113
Cell line name:	293T OS8 Cell Line
Gene ID/Accession #:	NA
Host cell line	293T
Cell type:	Human embryonic kidney
Description:	HEK293T cell line stable expressing membrane OKT3 scFV sequence
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 + 50ug/ml Hygromycin
Selection marker:	Hygromycin
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

293T-OS8 stable cell was used as an artificial antigen presenting cells (APCs), OS8 could function as a membrane anchored T cell engager that directly activates TCR in T cell-based assay.

III. Cell Line Generation

293T OS8 cell line was generated using lentiviral vector expressing a ScFV sequence of anti-human CD3 mAb OKT3 and a C-terminal domain of mouse CD8a which consist of transmembrane and cytoplasmic domains.

IV. Characterization using FACS

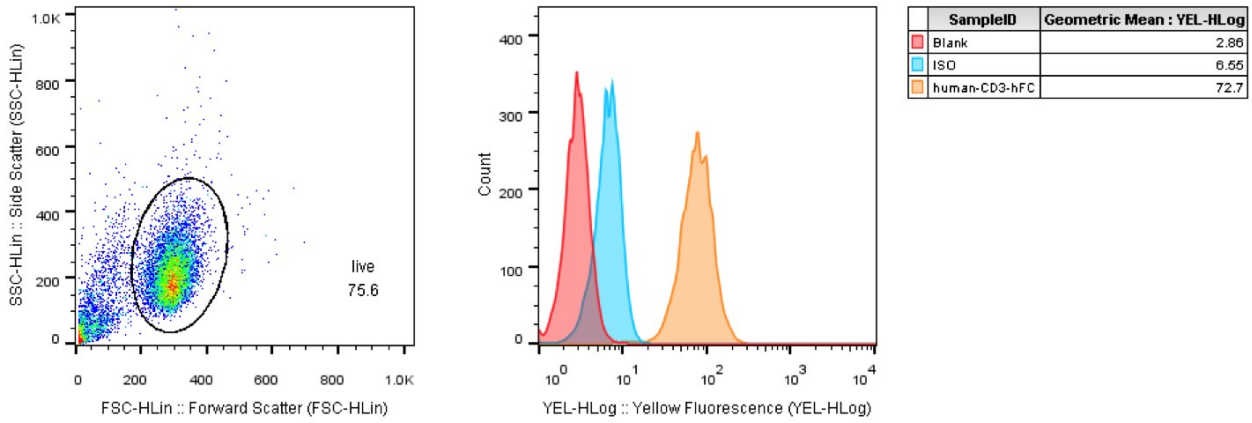

Figure: Characterization of OS8 overexpressing in 293T stable clones using FACS.

V. Application

Hybridoma or Binders of ligand screening in T cell activation assay

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. 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. Latouche, Jean-Baptiste; Sadelain, Michel (2000). "Induction of human cytotoxic T lymphocytes by artificial antigen-presenting cells". Nature Biotechnology. 18 (4): 405–409.

2. Perica, Karlo; Kosmides, Alyssa K; Schneck, Jonathan P (2015). "Linking form to function: Biophysical aspects of artificial antigen presenting cell design". Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 1853 (4): 781–790

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