293T human OX40 Cell Line

Cat. No: KC-0282 Version 17103102

Ⅰ. Cell Line Information

Catalog number	KC-0282
Cell line name:	293T human OX40 Cell Line
Gene ID/Accession #:	NM_003327.3
Host cell line	293T
Cell type:	Human embryonic kidney
Description:	HEK293T cell line stable expressing exogenous human OX40 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 ℃ with 5% CO_2
Doubling time:	Approximately 30 hours
Mycoplasma status:	Negative
Biosafety level:	1
Storage:	Liquid nitrogen immediately upon receiving

Ⅱ. Background

OX40, also named as CD134 or tumor necrosis factor receptor superfamily, member 4 (TNFRSF4), is a member of the TNFR superfamily acting as co-stimulatory immune checkpoint molecule expressed on the activated T cells. OX40 plays a critical role in maintaining of immune response and lead to a memory immune response. OX40-OX40 Ligand interaction is involved in allergic airway inflammation, graft-versus-host disease and autoimmune disease

and cardiovascular disease.

III. Cell Line Generation

293T human OX40 cell line was generated using lentiviral vector expressing human OX40 sequence.

IV. Characterization using FACS

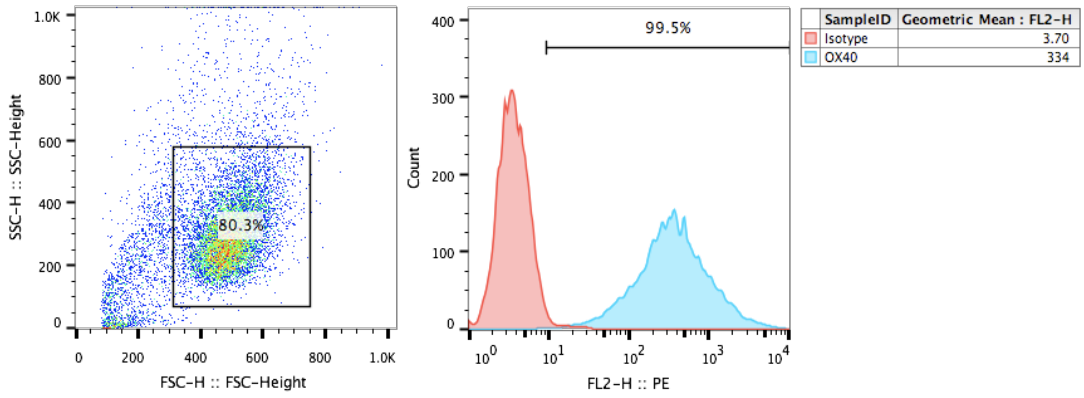

Figure: Characterization of OX40 overexpressing in 293T stable clones using OX40.

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℃ 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. 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. Ishii, Naoto, Takeshi Takahashi, Pejman Soroosh, and Kazuo Sugamura. 2010. “OX40–OX40 Ligand Interaction in T-Cell-Mediated Immunity and Immunopathology.” In, 105:63–98. Advances in Immunology. Elsevier. doi:10.1016/S0065-2776(10)05003-0.
2. Aspeslagh, Sandrine, Sophie Postel-Vinay, Sylvie Rusakiewicz, Jean-Charles Soria, Laurence Zitvogel, and Aurélien Marabelle. 2016. “Rationale for Anti-OX40 Cancer Immunotherapy.” European Journal of Cancer 52 (January). Elsevier: 50–66. doi:10.1016/j.ejca.2015.08.021.

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