

293T Cyno CD137 Cell Line

Cat. No: KC-0255

Version 17103102

I. Cell Line Information.....	1
II. Background.....	1
III. Cell Line Generation.....	2
IV. Characterization using FACS.....	2
V. Application.....	2
VI. Cell Resuscitation.....	2
VII. Cell Freezing.....	3
VIII. References.....	3

I. Cell Line Information

Catalog number	KC-0255
Cell line name:	293T cyno CD137 Cell Line
Gene ID/Accession #:	XM_005544888.2
Host cell line	293T
Cell type:	Human embryonic kidney
Description:	HEK293T cell line stable expressing exogenous cynomolgus CD137 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

CD137, also named 4-1BB ligand receptor and TNFRSF9, is a member of the tumor necrosis (TNF) receptor family and a famous co-stimulatory immune checkpoint molecule. CD137 is expressed on activated T cells, and nature killer cells, crosslinking of CD137 by agonistic antibody or its ligand enhance T cell proliferation, IL2 secretion and cytolytic activities.

III. Cell Line Generation

293T cyno CD137 cell line was generated using lentiviral vector expressing cynomolgus CD137 sequence.

IV. Characterization using FACS

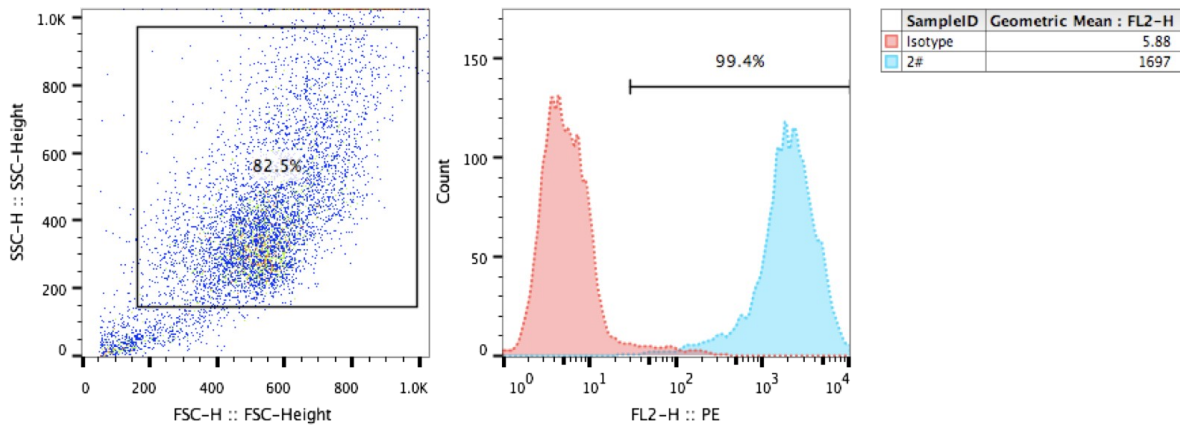


Figure: Characterization of CD137 overexpressing in 293T stable clones using FACS.

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% CO₂ incubator.
8. Split saturated culture 1:4 ~ 1:5 every 2~3 days; seed out at about 1-3 x 10⁵ 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 3×10^6 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°C freezer.
8. Transfer vials to liquid nitrogen for long-term storage.

VIII. References

1. Wolfi, M, J Kuball, W Y Ho, H Nguyen, T J Manley, M Bleakley, and P D Greenberg. 2007. "Activation-Induced Expression of CD137 Permits Detection, Isolation, and Expansion of the Full Repertoire of CD8+ T Cells Responding to Antigen Without Requiring Knowledge of Epitope Specificities." *Blood* 110 (1). American Society of Hematology: 201-10. doi:10.1182/blood-2006-11-056168.
2. Litjens, N H R, E A de Wit, C C Baan, and M G H Betjes. 2013. "Activation-Induced CD137 Is a Fast Assay for Identification and Multi-Parameter Flow Cytometric Analysis of Alloreactive T Cells." *Clinical and Experimental Immunology* 174 (1): 179-91. doi:10.1111/cei.12152.

Kyinno Biotechnology Co., Ltd.

Yizhuang Biomedical Park, No. 88, Beijing, China

Tel: +86-10-58222702

E-mail: bd@kyinno.com

Web: www.kyinno.com

For Research Use Only