293T hCSF1R Cell Line

Cat. No: KC-1220 Version 17031002

I. Cell Line Information

Catalog number	KC-1220
Cell line name:	293T hCSF1R Cell Line
Gene ID/Accession #:	NM_005211.2
Host cell line	293T
Cell type:	Human embryonic kidney
Description:	HEK293T cell line stable expressing exogenous human CSF1R gene
Quantity:	One vial of frozen cells (5X106 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 105 cells/ml
Incubation:	37 °C with 5% CO2
Doubling time:	Approximately 30 hours
Mycoplasma status:	Negative
Biosafety level:	1
Storage:	Liquid nitrogen immediately upon receiving

II. Background

Colony Stimulating factor 1 receptor (CSF1R), also named as macrophage colony-stimulating factor receptor (M-CSFR) and CD115, is a single pass type I membrane protein and functions as receptor for the cytokine of colony stimulating factor 1 (CSF1), the interaction of both molecules controls the production, differentiation and function of macrophages, and is also involved in the development and carcinogenesis of the mammary gland. Mutations in CSF1R are also associated with CML and type M4 AML. The identification of CSF1R as drug target has led to repaid development of the inhibitor of CSF1R in the treatment of cancer or inflammatory diseases, such as Pexidartinib, PLX7486, ARRY-382, JNJ-40346527, BLZ945, Emactuzumab, AMG820, IMC-CS4 and cabiralizumab.

III. Cell Line Generation

293T hCSF1R cell line was generated using lentiviral vector expressing human CSF1R sequence.

IV. Characterization using FACS

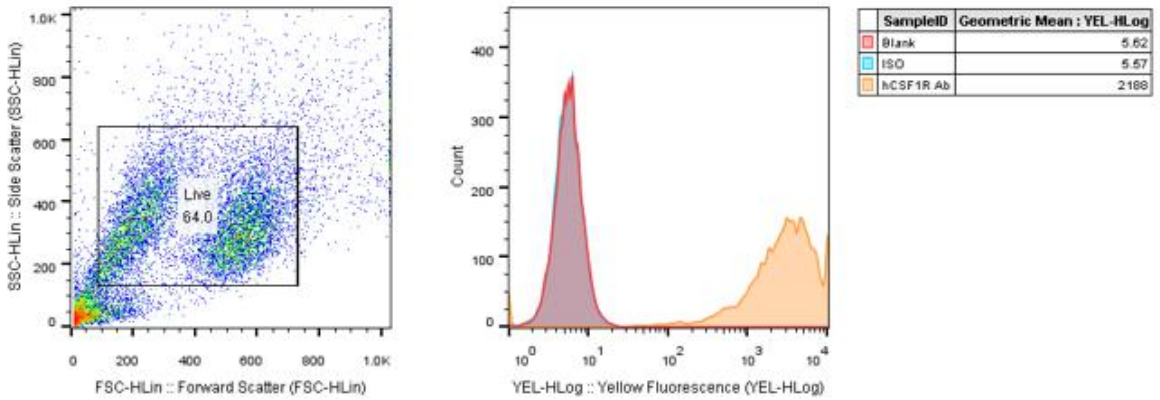

Figure: Characterization of CSF1R overexpressing in 293T stable clones using FACS.

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. Irvine, Katharine M, Christopher J Burns, Andrew F Wilks, Stephen Su, David A Hume, and Matthew J Sweet. 2006. “A CSF-1 Receptor Kinase Inhibitor Targets Effector Functions and Inhibits Pro-Inflammatory Cytokine Production from Murine Macrophage Populations.” The FASEB Journal 20 (11): 1921–23.
2. Ries, Carola H, Michael A Cannarile, Sabine Hoves, Jörg Benz, Katharina Wartha, Valeria Runza, Flora Rey-Giraud, et al. 2014. “Targeting Tumor-Associated Macrophages with Anti-CSF-1R Antibody Reveals a Strategy for Cancer Therapy.” Cancer Cell 25 (6). Elsevier Inc.: 846–59.

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