CHO-K1 Rabbit CD47 Cell Line

Cat. No: KC-1230 Version 19022601

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I. Cell Line Information

Catalog number KC-1230

Cell line name: CHO-K1 Rabbit CD47 Cell Line

Gene ID/Accession #: XM_008266994.2

Host cell line CHO-K1

Cell type: Chinese hamster ovary cell line

Description: CHOK1 cell line stable expressing exogenous Rabbit CD47 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: F12K + 10% FBS + 6ug/ml Puromycin

Selection marker: Puromycin

Morphology: Epithelial

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 24 hours

Mycoplasma status: Negative

Biosafety level: 1

Storage: Liquid nitrogen immediately upon receiving

II. Background

CD47, also named as integrin associated protein (IAP) is a transmembrane protein belonging to the immunoglobulin superfamily, which acts as a “don’t eta me” signal to macrophage after binding with its ligand signal-regulatory protein alpha (SIRPa), and is potential therapeutic target in some cancer and treatment of pulmonary fibrosis.

III. Cell Line Generation

CHO-K1 Rabbit CD47 cell line was generated using lentiviral vector expressing rabbit CD47 sequence.

IV. Characterization using FACS

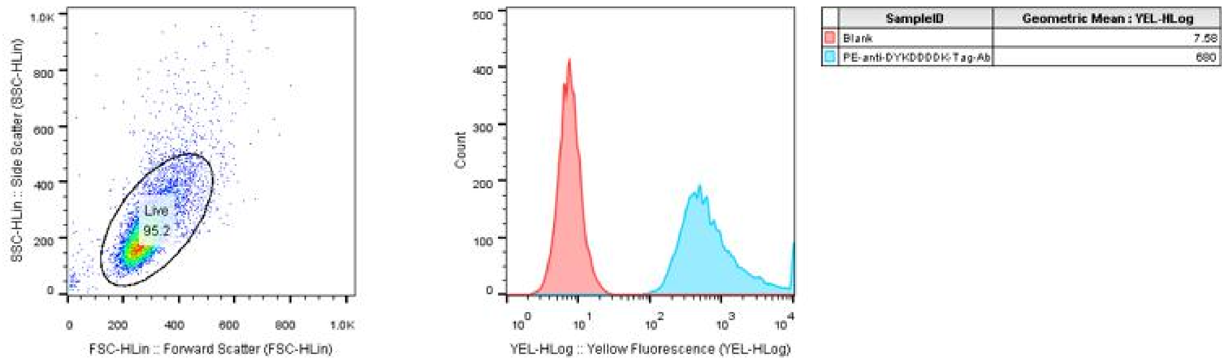

Figure: Characterization of CD47 overexpressing in CHOK1 stable clones using intracellular FACS.

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. Willingham, Stephen B, Jens-Peter Volkmer, Andrew J Gentles, Debashis Sahoo, Piero Dalerba, Siddhartha S Mitra, Jian Wang, et al. 2012. "The CD47-Signal Regulatory Protein Alpha (SIRPa) Interaction Is a Therapeutic Target for Human Solid Tumors.." Proceedings of the National Academy of Sciences of the United States of America 109 (17). National Acad Sciences: 6662–67. doi:10.1073/pnas.1121623109.

2. Vonderheide, Robert H. 2015. "CD47 Blockade as Another Immune Checkpoint Therapy for Cancer." Nature Medicine 21 (10). Nature Publishing Group: 1122–23. doi:10.1038/nm.3965.

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