

CHO-K1 Dog CD47 Cell Line

Cat. No: KC-1310

Version 19061901

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I. Cell Line Information

Catalog number	KC-1310
Cell line name:	CHO-K1 Dog CD47 Cell Line
Gene ID/Accession #:	NM_001080721.1
Host cell line	CHO-K1
Cell type:	Chinese hamster ovary cell line
Description:	CHOK1 cell line stable expressing exogenous dog 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% F12K + 20% FBS + 10% DMSO
Propagation medium:	F12K + 10% FBS + 5ug/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 Dog CD47 cell line was generated using lentiviral vector expressing dog CD47 sequence.

IV. Characterization using FACS

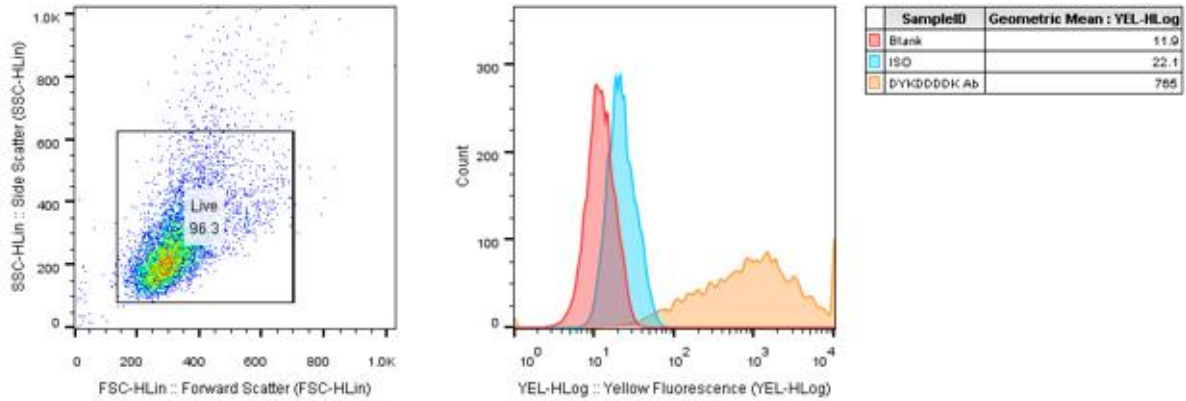


Figure: Characterization of dog CD47 overexpressing in CHOK1 stable clones using intracellular FACS.

V. Application

Hybridoma or Binders of ligand screening with FACS.

VI. Cell Resuscitation

1. Prewarm culture medium (F12K supplemented with 10% FBS and 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% F12K + 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 x 10⁶ 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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