

CHO-K1 human PVRL4 Cell Line

Cat. No: KC-1522

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I. Cell Line Information

Catalog number	KC-1522
Cell line name:	CHOK1 human PVRL4Cell Line
Gene ID/Accession #:	NM_030916.3
Host cell line	CHO-K1
Cell type:	Chinese hamster ovary cell line
Description:	CHOK1 cell line stable expressing exogenous human INSR 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 + 6ug/ml Puromycin
Selection marker:	Puromycin
Morphology:	Fibroblastoid cells growing as monolayer
Subculture:	Split saturated culture 1:4~1:8 every 2~3 days; seed out at about 1-2 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

PVRL4, also named nectin4, is a type I transmembrane glycoprotein belonging to the nectin family of Ig superfamily. PVRL4 is found upregulation in many human ductal breast, non-small cell lung and other carcinomas. In 2019, the PVRL4-directed antibody drug conjugate, enfortumab vedotin-ejfv was approved by the FDA to treat metastatic urothelial cancer.

III. Cell Line Generation

CHOK1 human PVRL4 cell line was generated using lentiviral vector expressing human PVRL4 sequence.

IV. Characterization using FACS

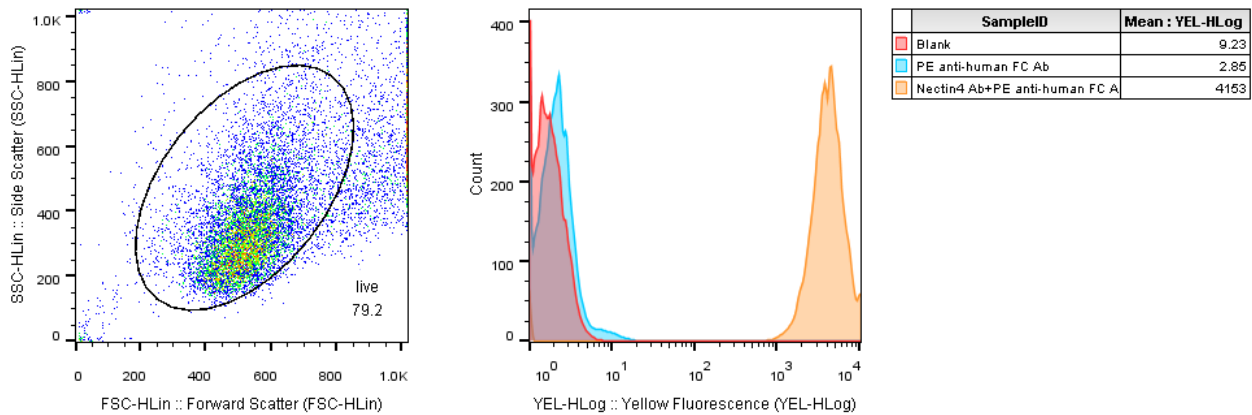


Figure: Characterization of PVRL4 overexpressing in CHOK1 stable clones using FACS.

V. Application

Hybridoma or Binders of ligand screening with FACS.

VI. Cell Resuscitation

1. Prewarm culture medium (F12K supplemented with 10% FBS and 6ug/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 ~ 1:8 every 2~3 days; seed out at about 1-2 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. Trypsin and 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. Challita-Eid, P. M. et al. Enfortumab vedotin antibody-drug conjugate targeting nectin-4 is a highly potent therapeutic agent in multiple preclinical cancer models. *Cancer Res.* 76, 3003–3013 (2016).

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