

## KATOIII Human CLDN18.2 Cell Line

Cat. No: KC-1453

Version 19032601

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### I. Cell Line Information

<b>Catalog number</b>	KC-1453
<b>Cell line name:</b>	KATOIII human CLDN18.2 cell line
<b>Gene ID/Accession #:</b>	NM_001002026.2
<b>Host cell line</b>	KATOIII
<b>Cell type:</b>	Human gastric cancer cell line
<b>Description:</b>	KATOIII cell line stable expressing exogenous human CLDN18 gene isoform 2
<b>Quantity:</b>	One vial of frozen cells (5X10 <sup>6</sup> per vial)
<b>Stability:</b>	Stable in culture over a minimum of 10 passages
<b>Application:</b>	Drug screening and biological assays
<b>Freeze medium:</b>	70% RPMI-1640 + 20% FBS + 10% DMSO
<b>Propagation medium:</b>	RPMI-1640 + 10% FBS + 1ug/ml puromycin
<b>Selection marker:</b>	Puromycin
<b>Morphology:</b>	Fibroblastoid cells growing as monolayer
<b>Subculture:</b>	Split saturated culture 1:2~1:3 every 2~3 days; seed out at about 2-4 x 10 <sup>5</sup> cells/ml
<b>Incubation:</b>	37 °C with 5% CO <sub>2</sub>
<b>Doubling time:</b>	NA
<b>Mycoplasma status:</b>	Negative
<b>Biosafety level:</b>	1
<b>Storage:</b>	Liquid nitrogen immediately upon receiving

### II. Background

CLDN18.2 is the isoform 2 of the claudin 18 gene, which belong to the larger claudin family and play the important role in cell tight junction in epithelial cells. CLND18.2 is found overexpressed on gastrointestinal adenocarcinoma and pancreatic tumors. The identification as a tumor target of CLDN18.2 has led to the repaid progress of antibody treatment of gastrointestinal adenocarcinoma and pancreatic tumors, such as IMAB362 (Claudiximab).

### III. Cell Line Generation

KATOIII CLDN18.2 cell line was generated using lentiviral vector expressing human CLDN18.2 sequence.

### IV. Characterization using FACS

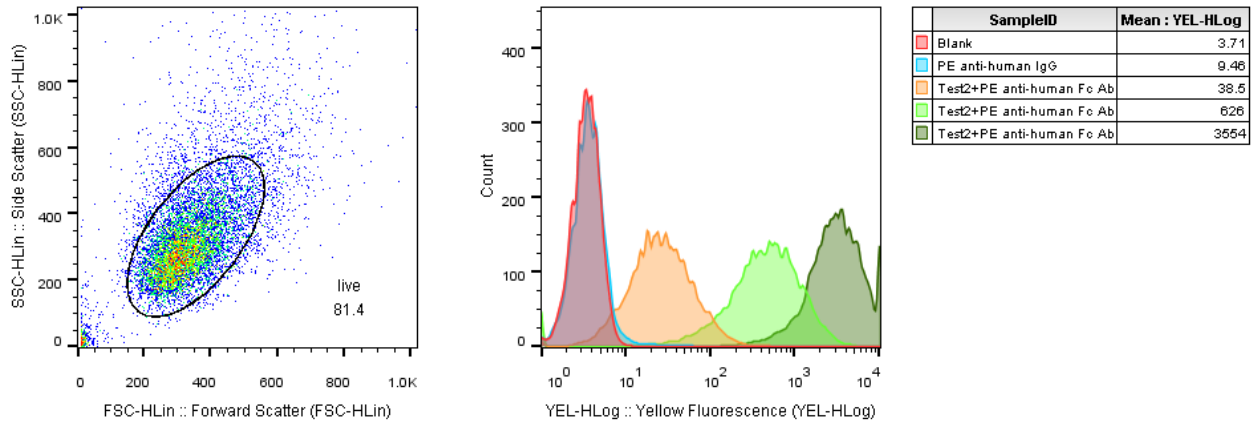


Figure: Characterization of CLDN18.2 overexpressing in KATOIII stable clones using FACS.

### V. Application

Hybridoma or Binders of ligand screening with FACS.

### VI. Cell Resuscitation

1. Prewarm culture medium (RMPI1640 supplemented with 10% FBS and 1ug/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<sub>2</sub> incubator.
8. Split saturated culture 1:4 ~ 1:5 every 2~3 days; seed out at about 1-3 x 10<sup>5</sup> 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  $3 \times 10^6$  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  $-80^{\circ}\text{C}$  freezer.
8. Transfer vials to liquid nitrogen for long-term storage.

## VIII. References

1. Micke, Patrick, Johanna Sofia Margareta Mattsson, Karolina Edlund, Miriam Lohr, Karin Jirström, Anders Berglund, Johan Botling, et al. 2014. "Aberrantly Activated Claudin 6 and 18.2 as Potential Therapy Targets in Non-Small-Cell Lung Cancer." *International Journal of Cancer* 135 (9): 2206–14. doi:10.1002/ijc.28857.
2. Singh, Prabhsimranjot, Sudhamshi Toom, and Yiwu Huang. 2017. "Anti-Claudin 18.2 Antibody as New Targeted Therapy for Advanced Gastric Cancer," May. *Journal of Hematology & Oncology*, 1–5. doi:10.1186/s13045-017-0473-4.
3. Sahin, U, M Koslowski, K Dhaene, D Usener, G Brandenburg, G Seitz, C Huber, and O Tureci. 2008. "Claudin-18 Splice Variant 2 Is a Pan-Cancer Target Suitable for Therapeutic Antibody Development." *Clinical Cancer Research* 14 (23): 7624–34. doi:10.1158/1078-0432.CCR-08-1547.

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