

## CT26.WT Human Siglec15 Cell Line

Cat. No: KC-1483

Version 19010101

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### I. Cell Line Information

|                             |   |
|-----------------------------|---|
| <b>Catalog number</b>       | KC-1483   |
| <b>Cell line name:</b>      | CT26.WT human siglec15 cell line                                  |
| <b>Gene ID/Accession #:</b> | NM_213602.2   |
| <b>Host cell line</b>       | CT26.WT   |
| <b>Cell type:</b>           | Mouse colon adenocarcinoma cell line                              |
| <b>Description:</b>         | CT26.WT cell line stable expressing exogenous human siglec15 gene |
| <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% RPMI1640 + 20% FBS + 10% DMSO                                 |
| <b>Propagation medium:</b>  | RPMI1640 + 10% FBS + 10ug/ml puromycin                            |
| <b>Selection marker:</b>    | Puromycin   |
| <b>Morphology:</b>          | Fibroblastoid cells growing as monolayer                          |
| <b>Subculture:</b>          | Split saturated culture 1:4~1:10 every 2~3 days                   |
| <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

Siglec-15 is a transmembrane glycoprotein, which belongs to the immunoglobulin superfamily and SIGLEC (sialic acid binding Ig-like lectin) family, and is found primarily on the surface of immune cells. Siglec-15 is that recognizes sialylated glycans and regulates osteoclast differentiation. Siglec-15 is a potential therapeutic target for osteoporosis and plays a conserved regulatory role in the immune system of vertebrates.

### III. Cell Line Generation

CT26.WT human siglec15 cell line was generated using lentiviral vector expressing human siglec15 sequence.

### IV. Characterization using FACS

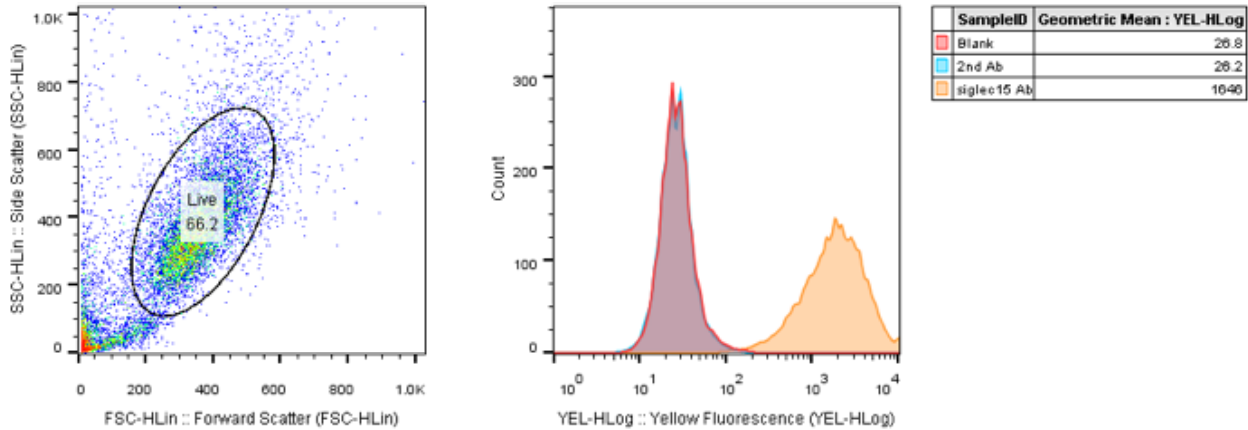


Figure: Characterization of siglec15 overexpressing in CT26.WT stable clones using FACS.

### V. Application

Hybridoma or Binders of ligand screening with FACS.

### VI. Cell Resuscitation

1. Prewarm culture medium (RPMI1640 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 x 10<sup>5</sup> cells/ml.

### VII. Cell Freezing

1. Prepare the freezing medium (70% RPMI1640 + 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. Wang, Jun, Jingwei Sun, Linda N Liu, Dallas B Flies, Xinxin Nie, Maria Toki, Jianping Zhang, et al. 2019. "Siglec-15 as an Immune Suppressor and Potential Target for Normalization Cancer Immunotherapy." Nature Medicine, March. Springer US, 1–32. doi:10.1038/s41591-019-0374-x.

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