

# CT26.WT human B7H4 Cell Line

Cat. No: KC-1002

Version 18051101

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## I. Cell Line Information

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|-----------------------------|---|
| <b>Catalog number</b>       | KC-1002   |
| <b>Cell line name:</b>      | CT26.WT human B7H4 Cell Line                                  |
| <b>Gene ID/Accession #:</b> | NM_024626.3   |
| <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 B7H4 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 + 1ug/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

B7H4, also named as B7x and B7S1, is transmembrane glycosylated protein belonging to B7 family of immunomodulatory proteins. B7H4 is expressed on the surface of many immune cells, including activated lymphocytes, macrophages, monocytes, dendritic cells, it can dampen T cells response and induces cell cycle arrest of T cells.

### III. Cell Line Generation

CT26.WT human B7H4 cell line was generated using lentiviral vector expressing human B7H4 sequence.

### IV. Characterization using FACS

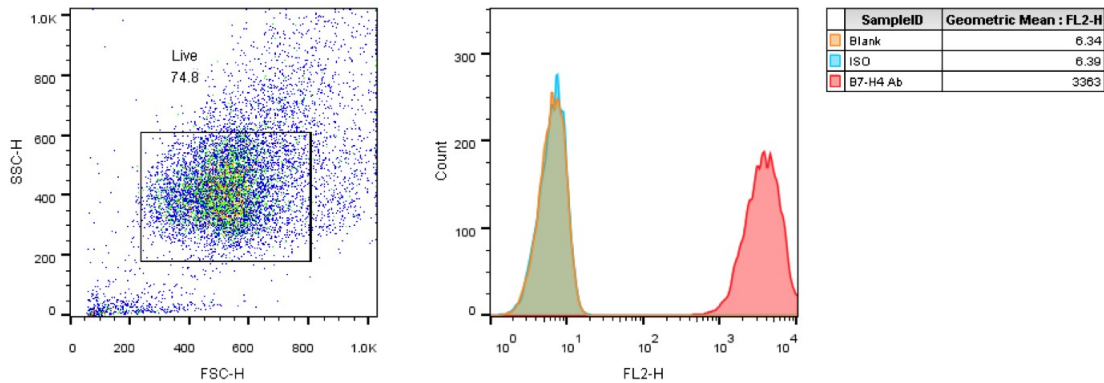


Figure: Characterization of B7H4 overexpressing in CT26.WT stable clones using FACS.

### 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. Sica, Gabriel L, In-Hak Choi, Gefeng Zhu, Koji Tamada, Sheng-Dian Wang, Hideto Tamura, Andrei I Chapoval, Dallas B Flies, Jürgen Bajorath, and Lieping Chen. 2003. "B7-H4, a Molecule of the B7 Family, Negatively Regulates T Cell Immunity." *Immunity* 18 (6): 849–61. doi:10.1016/S1074-7613(03)00152-3.
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3. Podojil, Joseph R, and Stephen D Miller. 2017. "Potential Targeting of B7-H4 for the Treatment of Cancer." *Immunological Reviews* 276 (1). Wiley/Blackwell (10.1111): 40–51. doi:10.1111/imr.12530.

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