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# Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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# Data Sheet

## CTLA4 / IL-2 Reporter - Jurkat Recombinant Cell Line
## Catalog #: 79525

**Product Description**
Recombinant Jurkat T cell expressing firefly luciferase gene under the control of IL-2 promoter with constitutive expression of human CTLA4 (Cytotoxic T-Lymphocyte Associated Protein, CD152; GenBank Accession #NM_005214).

**Background**

CTLA4, also known as CD152, is a protein receptor that functioning as an immune checkpoint. It is expressed by activated T cells and transmits an inhibitory signal to T cells. CTLA4 is homologous to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells. CTLA-4 binds to CD80 and CD86 with greater affinity and avidity than CD28 thus enabling it to outcompete CD28 for its ligands and act as an "off" switch when bound to CD80 or CD86. CTLA-4 is an important immunotherapy target for the treatment of cancer and autoimmune diseases.

**Application**
- Screen for activators or inhibitors of CTLA4 signaling in a cellular context
- Characterize the biological activity of CTLA4 and its interactions with ligands

**Format**
Each vial contains 2 x $10^6$ cells in 1 ml of 10% DMSO

**Storage**
Immediately upon receipt, store in liquid nitrogen.

**Mycoplasma Testing**
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of *Mycoplasma* species.

**General Culture Conditions**

**Thaw Medium 2 (BPS Cat. #60184):** RPMI1640 medium (Life Technologies #A10491-01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01)
**Growth Medium 2A (BPS Cat. #60190):** Thaw Medium 2 (BPS Cat. #60184) plus 1 mg/ml of Geneticin (Life Technologies #11811031) and 200 µg/ml of Hygromycin B (Life Technologies #10687-010).

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Cells should be grown at 37°C with 5% $CO_2$ using Growth Medium 2A (BPS Bioscience #60190) (Thaw Medium 2 BPS Cat. # 60184 plus Geneticin and Hygromycin B).

**To thaw the cells,** it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 (**no Geneticin and Hygromycin B)**. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 2 **(no Geneticin and Hygromycin B)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% $CO_2$ incubator. After 24 hours of culture, add an additional ~3 ml of Thaw Medium 2 (**no Geneticin and Hygromycin B)**, and continue growing culture in a $CO_2$ incubator at 37°C until the cells are ready to be split. Cells should be split before they reach ~2.5x$10^6$ cells/ml. At first passage, switch to Growth Medium 2A **(contains Geneticin and Hygromycin B).**

**To passage the cells**, dilute cell suspension into new culture vessels at no less than 0.2x$10^6$ cells/ml. Subcultivation ratio: 1:5 to 1:10 twice a week.

*Note: Just after thawing, the cells may grow at a slower rate. It is recommended to split the cells at no less than 0.4* x$10^6$ cells/ml *at the beginning of culturing. After ~two passages, the cell growth rate increases and the cells can be split to 0.2x$10^6$ cells/ml.*

**To freeze down the cells**, spin down cells, and resuspend cell pellet in 4°C Freezing Medium (10% DMSO + 90% FBS) to ~2x$10^6$ cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.
It is recommended to expand the cells and at early passage freeze down more than 10 vials of cells for future use.

**Functional Validation and Assay Performance**
Expression of human CTLA4 in CTLA4/IL-2 Reporter-Jurkat cell line was confirmed by FACS.

The functionality of the cell line was validated using a CTLA4 cell-based assay. In this assay, CTLA4/IL-2 Reporter-Jurkat cells are used as effector cells; Raji cells are used as target cells. When these two cells are co-cultivated, addition of anti-CD3 antibody results in the expression of the IL-2 luciferase reporter in effector cells, however, the costimulatory signal from the CD28 on the effector cells are suppressed by the expression of CTLA4 due to the higher binding affinity between CTLA4 and CD28 ligands, CD80 and CD86. CTLA4 neutralizing antibody blocks CTLA4: CD80/86 interactions and releases the costimulatory signal from the CD28 and its ligands.

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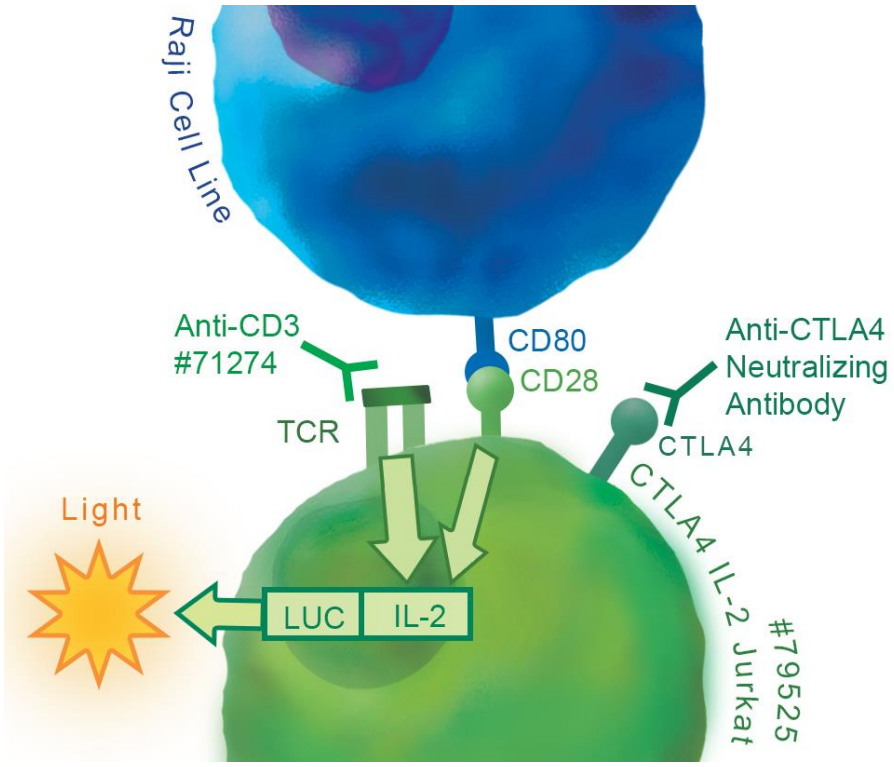


**Materials Required but Not Supplied**

- Raji cell (ATCC # CCL-86)
- Thaw Medium 2 (BPS Bioscience #60184)
- Growth Medium 2A (BPS Bioscience #60190)
- Assay medium: RPMI1640 medium (Life Technologies #A10491-01)
- Anti-CD3 agonist antibody (BPS bioscience #71274)
- Anti-CTLA4 neutralizing antibody (Biolegend #349904, clone#L3D10)
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS bioscience # 60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

1. Harvest CTLA4/IL-2 Reporter-Jurkat cells by centrifugation and resuspend in serum free assay medium. Dilute the cells to $2.4 \times 10^6$ / ml in assay medium.

2. Prepare serial dilution of anti-CTLA4 antibody in assay medium (the concentration of antibody here is 10x of the final treatment concentration of antibody).

3. Harvest Raji cells by centrifugation and resuspend in assay medium. Dilute cells to $0.6 \times 10^6$ / ml in assay medium. Add anti-CD3 agonist antibody into Raji cells to the final concentration of 200 ng/mL.

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4. Add 50 µl of CTLA4/IL-2 Reporter- Jurkat cells ($2.4x10^6$ / ml) and 10 µl of diluted anti-CTLA4 antibody per well in 96-well white clear-bottom assay plate. Preincubate for 15 to 30 min. After incubation, add 40 µl of Raji cells/anti-CD3 agonist antibody mixture per well. Tap the plate gently to mix.

   Final cell density of CTLA4/IL-2 Reporter- Jurkat cells and Raji cells is $1.2 x10^5$ /well and $0.24 x10^5$ /well, respectively. Final concentration of anti-CD3 antibody is 80 ng/ml. Set up each treatment in at least triplicates.

   Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

   Incubate the plates at 37° in a CO2 incubator for 20 hours.

5. After 20 hours incubation, perform luciferase assay using the ONE-Step luciferase assay system: Add 100 µl of One-Step Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.
   *If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.*

6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
   The fold induction of IL-2 luciferase reporter expression = background-subtracted luminescence of antibody treated well / average background-subtracted luminescence of untreated control wells.

**Figure 1. Characterization of biological activity of anti-CTLA4 neutralizing antibody in CTLA4 cell-based assay using the CTLA4/IL-2 Reporter-Jurkat cells co-cultured with Raji cells/anti-CD3 antibody.**

CTLA4/IL-2 Reporter-Jurkat cells (BPS bioscience #79525) (or control IL-2 Reporter – Jurkat cells, BPS Cat. #60481) were incubated with anti-CTLA4 neutralizing antibody (Biolegend #349904, clone#L3D10), Raji cells (ATCC # CCL-86), and anti-CD3 antibody (BPS bioscience #71274). After incubation, ONE-Step™ Luciferase reagent (BPS Cat. #60690) was added to the cells to measure IL-2 reporter activity.

The fold induction is equal to background-subtracted luminescence of antibody-treated well / background-subtracted luminescence of untreated-control wells of each respective cell line.

**A.** Anti-CTLA4 neutralizing antibody increased IL-2 luciferase reporter activity in CTLA4/IL-2 Reporter-Jurkat cells, but not IL-2 Reporter – Jurkat cells, when co-cultured with Raji cells/anti-CD3 antibody.

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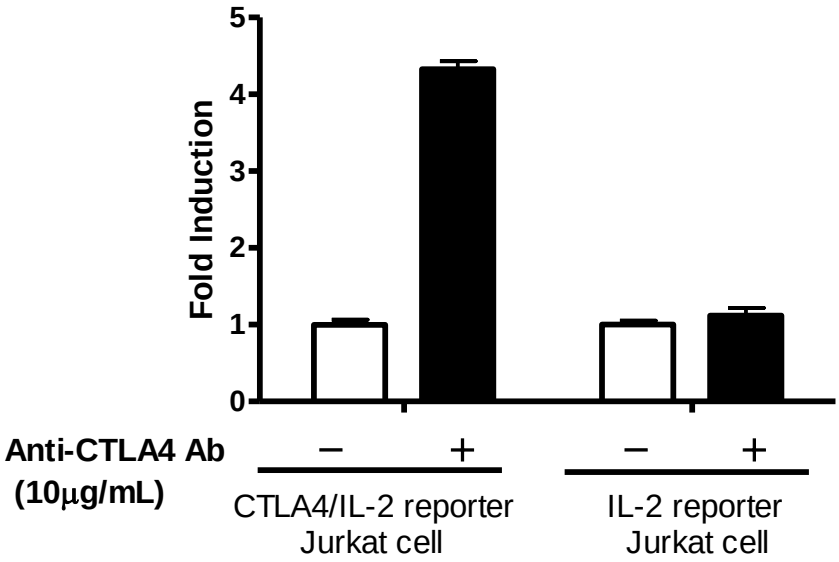


**B.** Dose response of anti-CTLA4 neutralizing antibody in CTLA4/IL-2 Reporter-Jurkat cells. Anti-PD-1 neutralizing antibody (BPS bioscience #71120) is shown as a negative control.

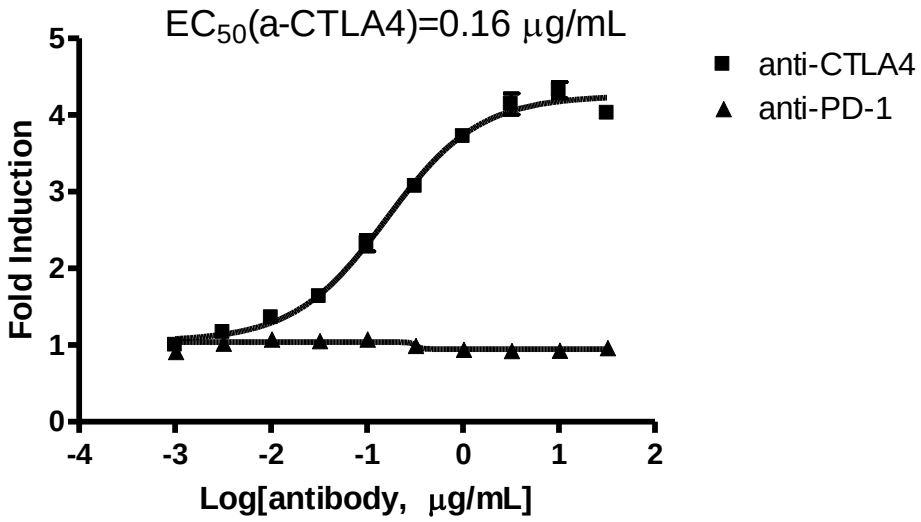

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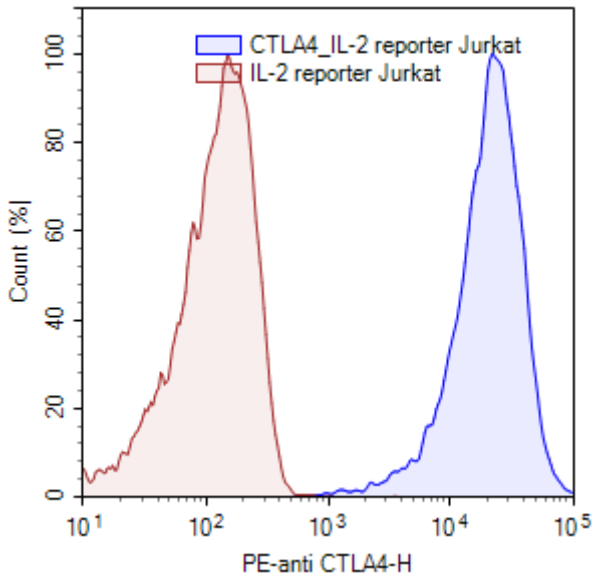
**Figure 2 FACS analysis of cell surface expression of CTLA4 in CTLA4/IL-2 Reporter-Jurkat cells.**
CTLA4/IL-2 Reporter-Jurkat or IL-2 Reporter-Jurkat cells were stained with PE-labeled anti-CTLA4 antibody (Biolegend #349906) and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.



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## Related Products

| Product | Cat. # | Size |
|---|---|---|
| IL-2 Reporter – Jurkat cell line | 60481 | 2 vials |
| Anti-CD3 antibody | 71274-2 | 100 µg |
| Anti-CTLA4 neutralizing antibody | 71212 | 100 µg |
| ONE-Step™ Luciferase Assay System | 60690-1 | 10 ml |
| ONE-Step™ Luciferase Assay System | 60690-2 | 100 ml |
| PD-1/NFAT Reporter-Jurkat cell line | 60535 | 2 vials |

## Notes

License Disclosure: Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit the use of this cell line in humans or for therapeutic or drug use. The license does not permit modification of the cell line in any way. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. BPS does not warrant the suitability of the cell line for any particular use, and does not accept any liability in connection with the handling or use of the cell line. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees; contact sales@bpsbioscience.com for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.