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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

CD27/NF-κB Reporter-Jurkat Recombinant Cell Line
Catalog #: 79509

Product Description
Recombinant Jurkat T cell expressing firefly luciferase gene under the control of NF-κB response elements with constitutive expression of human CD27 (also known as Tumor Necrosis Factor Receptor Superfamily Member 7, TNFRSF7, and T14, NM_001242)

Background
CD27 is a member of the tumor necrosis factor (TNF) receptor superfamily, which includes the T cell co-stimulatory receptors OX40, 4-1BB and herpesvirus entry mediator (HVEM). CD27 is expressed on various types of T cells, B cells and a subset of natural killer cells. It activates NF-κB and MAPK/JNK signaling upon interaction with its TNF-like ligand, CD70, which is expressed by numerous tumor cells. Adaptor proteins TRAF2 and TRAF5 can also stimulate CD27 signaling. Activation of CD27 leads to lymphoid proliferation, differentiation, apoptosis, and the induction of long-term memory. The CD27/CD70 pathway is a key target for the development of treatments for cancer and inflammatory diseases.

Application
- Screen for agonists or antagonists of CD27-CD70 signaling in a physiologically relevant cellular context
- Characterize T cell-mediated immune responses of CD27 and its interaction with CD70

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

Storage
Store in liquid nitrogen immediately upon receipt

Mycoplasma Testing
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination.

Thaw Medium 2 (BPS #60184): RPMI1640 medium (Life Technologies #A10491-01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01)

Growth Medium 2C (BPS #79592): Thaw Medium 2 (BPS Bioscience #60184), 1 mg/ml of Geneticin (Life Technologies #11811031), and 100 µg/ml of Hygromycin B (Hyclone #SV30070.01)

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Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2C (Thaw Medium 2, Geneticin, and Hygromycin B).

Recommended Culture Condition

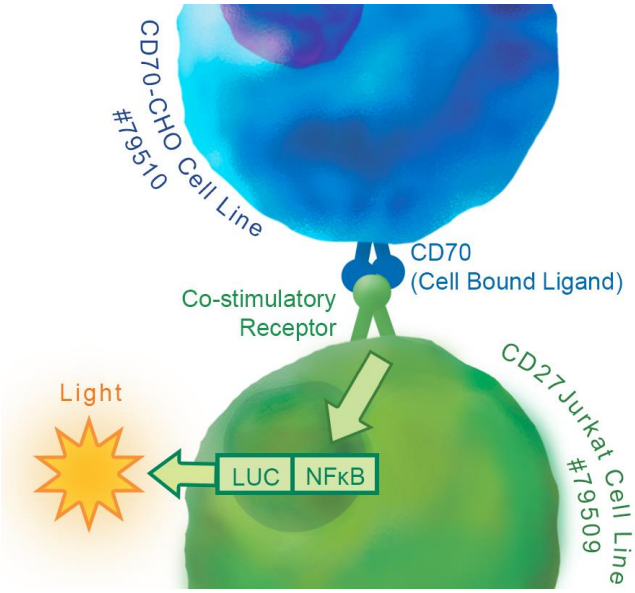
It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 **(**no Geneticin and Hygromycin B). Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 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 – 4 ml of growth medium without antibiotics. At first passage, switch to Growth Medium 2C (contains Geneticin and Hygromycin B). Cells should be split before they reach 2 x 10^6 cells/ml. Note: This cell line tends to grow more slowly than the parental Jurkat cells.

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

Functional Validation and Assay Performance

Expression of human CD27 in the Jurkat cell line was confirmed by Flow Cytometry. The functionality of the cell line was validated using a CD27:CD70 cell-based assay. In this assay, human CD27/NF-κB Reporter-Jurkat T cells are used as effector cells; CHO cells over-expressing human CD70 are used as target cells. When these two cells are co-cultivated, CD27 and CD70 ligation results in the expression of the NF-κB luciferase reporter. Anti-CD27 blocking antibody blocks CD27:CD70 interaction and diminishes the expression of the NF-κB luciferase reporter.

Assay Principle



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Materials Required but Not Supplied

- Assay Medium: Thaw Medium 2 (BPS Bioscience #60184)
- Growth Medium 2C (BPS Bioscience #79592)
- CD70-CHO K1 Recombinant Cell Line (BPS Bioscience #79510)
  - Thaw Medium 3, BPS Bioscience #60186
  - Growth Medium 3D (BPS Bioscience #79539)
- FcGR2B-CHO K1 Recombinant Cell Line (BPS Bioscience #79511)
  - Thaw Medium 3, BPS Bioscience #60186
  - Growth Medium 3D (BPS Bioscience #79539)
- 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

Assay Protocol and Functional Analysis

A) Functional assay of blocking antibody on CD27/NF-κB Reporter-Jurkat cells cocultured with CD70-CHO cells

1. Harvest CD70-CHO cells from culture in growth medium and seed cells at a density of 30,000 cells per well into white clear-bottom 96-well microplate in 100 µl growth medium. Incubate the plate at 37°C in a CO_2 incubator overnight.

2. Next day, harvest the CD27/NF-κB Reporter-Jurkat cells by centrifugation and resuspend in assay medium. Dilute the cells to 4 x10^5 / ml in assay medium.

   To test anti-CD27 blocking antibody, preincubate the CD27/NF-κB Reporter-Jurkat cells with anti-CD27 antibody for 15-30 min. After incubation, remove the medium from CD70-CHO cells and add 100 µl of CD27/NF-κB Reporter-Jurkat cells/anti-CD27 antibody mixture to the wells.

   To test anti-CD70 blocking antibody, remove the medium from CD70-CHO cells, add 50 µl of diluted anti-CD70 antibody to the wells and incubate for 15-30 min. After incubation, add 50 µl of CD27/NF-κB Reporter-Jurkat cells (8 x10^5 /ml) to the wells.

   The final cell density of CD27/NF-κB Reporter-Jurkat cells is ~4 x10^4 /well. Set up each treatment in at least triplicate.

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

3. Incubate the plate at 37°C in a CO_2 incubator for 5 hours.

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

5. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

   The fold induction of NF-κB luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Figure 1. The CD27/NF-κB Reporter Activities Stimulated by CD70-CHO Cells.

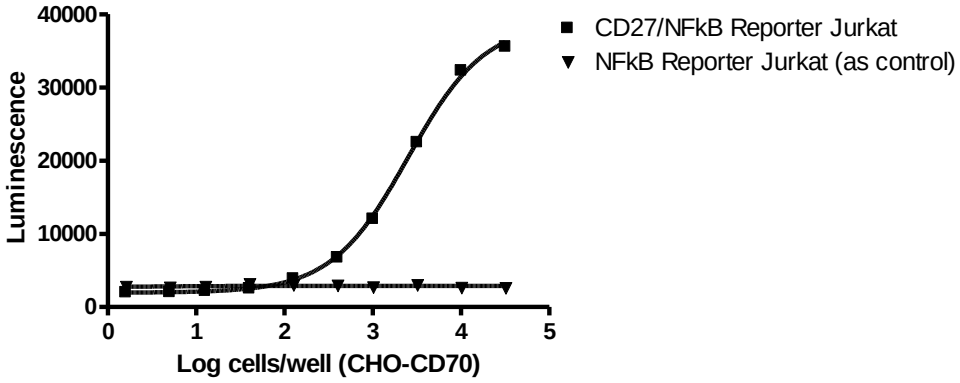

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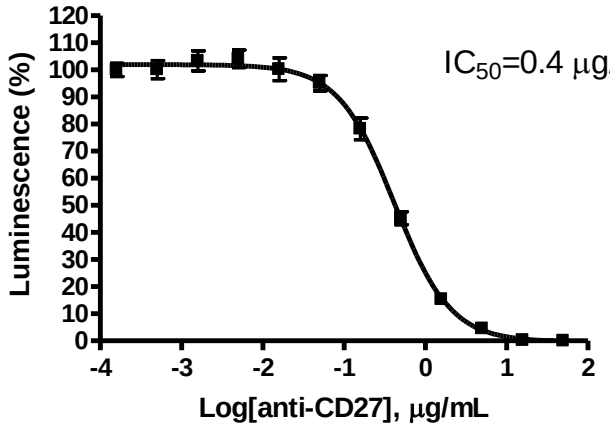
Figure 2. Anti-CD27 Blocking Antibody Dose Response in Co-Cultured CD27/NFκB Reporter-Jurkat and CD70-CHO cells.
The IC50 of anti-CD27 blocking antibody (Clone#57703, R&D systems #MAB382) is 0.4 µg/ml.



B) Functional assay of anti-CD27 agonist antibody on CD27/NF-κB Reporter-Jurkat cells cocultured with FcGR2B CHO K1 cells

1. Harvest CD27/NF-κB Reporter-Jurkat cells from culture in growth medium and seed 40,000 cells per well into white clear-bottom 96-well plate in 50 µl of assay medium (Thaw Medium 2).

2. Harvest FcGR2B CHO K1 cells and seed 80,000 cells/well in 50 µl of Thaw Medium 3 for coculture with the CD27/NF-κB Reporter-Jurkat cells.

3. Immediately dilute anti-CD27 agonist antibody (BPS cat #100111) in Thaw Medium 2 and treat cells with 10 µl of 10X dilutions of the anti-CD27 antibody.

4. Incubate the plate at 37°C in a CO_2 incubator for 16 hours.

5. Perform luciferase assay using the ONE-Step luciferase assay system: Add 100 µl of ONEStep Luciferase reagent per well (BPS Bioscience, #60690) and rock at room temperature for 20 minutes. Measure luminescence using a luminometer

   Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NF-κB luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

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Figure 3 Dose response of anti-CD27 agonist antibody on CD27/NF-κB Reporter-Jurkat cells cocultured with FcGR2B CHO K1 cells.
CD27/NF-κB Reporter-Jurkat cells (BPS Cat. #79509) were cocultured with FcGR2B-CHO cells (BPS Cat# 79511). Serial dilutions of anti-CD27 antibody were added to the cells, and then incubated at 37°C incubator for 16 hrs. After the treatment, the Luciferase assay was performed.

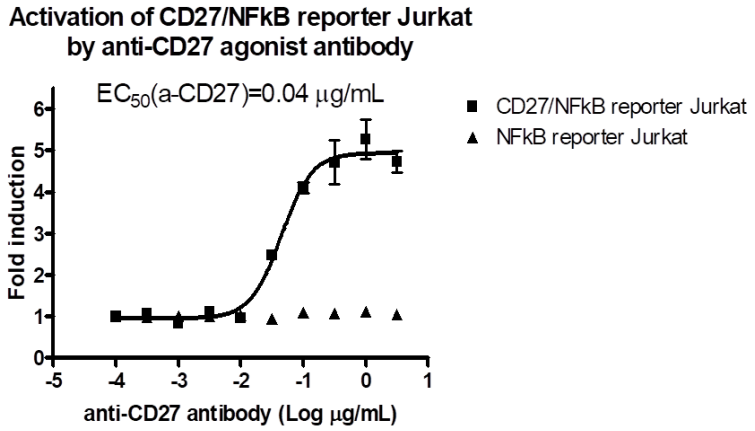
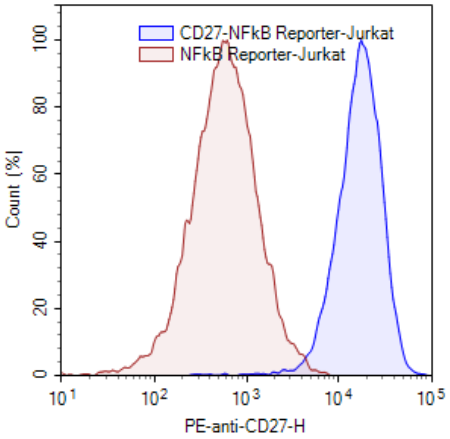


Figure 4. FACS Analysis of Cell Surface Expression of CD27 in CD27/NF-κB Reporter-Jurkat cells.
CD27/NF-κB Reporter-Jurkat cells or NF-κB Reporter-Jurkat cells were stained with PE-labeled anti-CD27 antibody (Biolegend, #356406; Clone#M-T271) and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.



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Sequence

Human CD27 (NM_001242; Genbank Accession #BC012160)

MARPHPWWLCVLGTLVGLSATPAPKSCPERHYWAQGKLCCQMCEPGTFLVKDCDQHRKAAQCDP
CIPGVSFSPDHHTRPHCESCRHCNSGLLVRNCTITANAECACRNGWQCRDKECTECDPLPNPSL
TARSSQALSPHPQPTHLPYVSEMLEARTAGHMQTLADFRQLPARTLSTHWPPQRSLCSSDFIRI
LVIFSGMFLVFTLAGALFLHQRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEP
ACSP

Related Products

Product	Cat. #	Size
CD27, Fc fusion Protein	71176	100 µg
CD70(CD27L), His-tag Protein	71178	100 µg
NF-κB Luciferase Reporter – Jurkat cell line	60651	2 vials
CD27 CHO-K1 Stable Recombinant Cell Line	60624	2 vials
CD70 CHO-K1 Stable Recombinant Cell Line	79510	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Thaw Medium 2	60184	100 ml
Thaw Medium 3	60186	100 ml
Growth Medium 2C	79592	500 ml
Anti-CD27 Agonist Antibody	100111	50 µg, 100 µg

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.