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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

LAG3 / IL-2 Reporter - Jurkat Recombinant Cell Line
Catalog #: 79813

Product Description
Recombinant Jurkat T cell expressing firefly luciferase gene under the control of IL-2 response elements with constitutive expression of human LAG3 (lymphocyte-activation gene 3, CD223, GenBank Accession # NM_002286).

Background
Lymphocyte-activation gene 3 (LAG3, CD223) is a cell surface protein that belongs to the immunoglobulin (Ig) superfamily. LAG3 is expressed on activated T cells, natural killer cells, B cells, and plasmacytoid dendritic cells. Its main ligand is MHC class II, to which it binds with higher affinity than CD4. It negatively regulates cellular proliferation, activation, and homeostasis of T cells in a similar fashion to CTLA-4 and PD-1, and has been reported to play a role in Treg suppressive function. A number of LAG3 antibodies are in preclinical development for treatments for cancer and autoimmune disorders. LAG3 may be a better immune checkpoint inhibitor target than CTLA-4 or PD-1 since antibodies to these two checkpoints are only activating effector T cells, and not inhibiting Treg activity while an antagonist LAG3 antibody can both activate effector T cells (by downregulating the LAG3 inhibiting signal) and inhibit induced (i.e. antigen-specific) Treg suppressive activity.

Application
- Screen for activators or inhibitors of LAG3 signaling in a cellular context
- Characterize the biological activity of LAG3 and its interactions with ligands

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

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing
The cell line has been screened using the luminescence-based Lonza MycoAlert™ Mycoplasma Detection Kit (Lonza, USA Catalog #: LT07-318) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

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

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Growth Medium 2J (BPS Bioscience #79812): Thaw Medium 2 (BPS Bioscience #60184) plus 500 µg/ml of Geneticin (Life Technologies #11811031) and 1 µg/ml of Puromycin Dihydrochloride (ThermoFisher #A1113803).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2J (BPS Bioscience #79812).

To thaw the cells, rapidly 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 Puromycin B)**. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 2 **(no Geneticin and Puromycin B)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator until the cells are ready to be split. Cells should be split before they reach ~2 x 10^6 cells/ml. At first passage, switch to Growth Medium 2J **(contains Geneticin and Puromycin B).**

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 twice a week. Cells should be split before they reach 2 x 10^6 cells/ml.

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 approximately two passages, the cell growth rate increases and the cells can be split to 0.2 x 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 ~2 x 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 at an early passage and freeze more than 10 vials of cells for future use.

Functional Validation and Assay Performance
Expression of human LAG3 in LAG3/IL-2 Reporter-Jurkat cell line was confirmed by FACS.

The functionality of the cell line was validated using a LAG3 cell-based assay. In this assay, LAG3/IL-2 Reporter-Jurkat cells are used as effector cells and TCR activator-Raji cells are used as target cells. When these two cells are co-cultivated, both CD3ζ and costimulatory CD28 pathways are activated, resulting the expression of IL-2 luciferase reporter in effector cells. However, the activation signal on the effector cells is suppressed by the expression of LAG3 due to the binding of the co-inhibitory receptor LAG3 with its ligand MHC class II molecules, which inhibits the T cell activation and the expression of IL-2-responsive luciferase in the effector cells. This inhibition can be specifically reversed by LAG3 neutralizing antibody and MHC II blocking antibody. LAG3 neutralizing antibody and/or MHC II antibody blocks LAG3: MHC class II interaction and promotes T cell activation, resulting in reactivation of the IL-2=responsive luciferase reporter.

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Materials Required but Not Supplied

- TCR activator-Raji cell (BPS Bioscience #60556)
- Assay medium: Thaw Medium 2 (BPS Bioscience #60184)
- Growth Medium 2J (BPS Bioscience #79812)
- Anti-LAG-3 neutralizing antibody (BPS Bioscience #71219)
- Anti-human MHC class II (HLA-DR), clone L243 (BioXCell #BE0306)
- 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 LAG3/IL-2-reporter Jurkat cells by centrifugation and resuspend in assay medium at 2.4 x 10^6cells/ml, pre-incubate with anti-LAG-3 antibody at 37°C in a CO_2 incubator for 30 to 60 minutes (the concentration of antibody here is 5x of the final treatment concentration of antibody).

2. Harvest Raji cells by centrifugation and resuspend in assay medium at 0.2 x 10^6 cells/ml. Preincubate the cells at 37°C in a CO_2 incubator for 30 minutes.

3. Combine 50 µl of LAG3/IL-2 Reporter Jurkat cells (2.4 x 10^6 cells/ml) and 50 µl of TCR activator-Raji cells (0.2 x 10^6 cells/ml) per well in 96-well white clear-bottom assay plate. Mix the plate gently. Leave a couple of wells empty for use as a cell-free control (below).

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   Final cell density of LAG-3/IL-2 Reporter- Jurkat cells and Raji cells is 12 x 10^4 cells/well and 1 x 10^4 cells/well, respectively. Final concentration of antibody is 1x. Final volume is 100 µl in each well. Set up each treatment in at least triplicate.

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

   Incubate the plates at 37°C in a CO_2 incubator for 24 hours.

4. After ~24 hours incubation, perform the luciferase assay using the ONE-Step™ luciferase assay system: Prepare the ONE-Step reagents as directed and add 100 µl of ONE-Step Luciferase reagent per well. Rock gently at room temperature for ~20 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 IL-2 luciferase reporter expression = background-subtracted luminescence of antibody treated well / average background-subtracted luminescence of untreated control wells.

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Figure 1. Characterization of biological activity of anti-LAG3 neutralizing antibody in LAG3 cell-based assay using the LAG3/IL-2 Reporter-Jurkat cells co-cultured with TCR activator-Raji cells.

LAG3/IL-2 Reporter-Jurkat cells (BPS Bioscience #79813) were incubated with anti-LAG3 neutralizing antibody (BPS Bioscience #71219) and TCR activator-Raji cells (BPS Bioscience #60556). After incubation, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure IL-2 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.

Dose response of anti-LAG3 neutralizing antibody in LAG3/IL-2 Reporter-Jurkat cells.

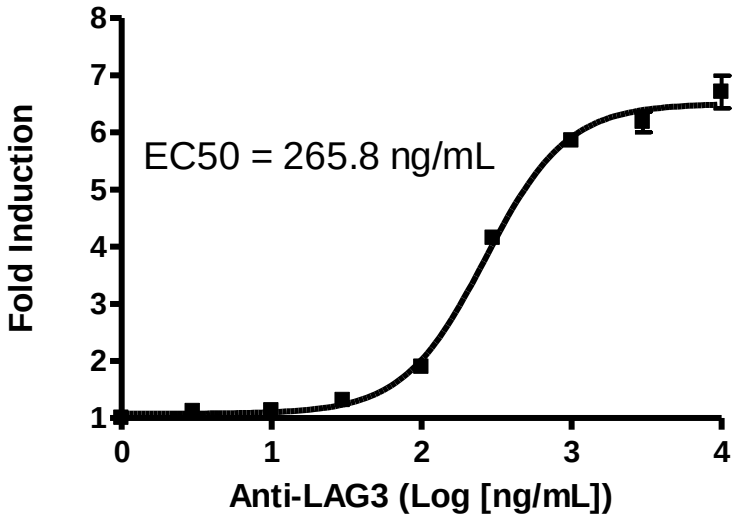

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Figure 2. Characterization of biological activity of anti-MHC class II antibody in LAG3 cell-based assay using the LAG3/IL-2 Reporter-Jurkat cells co-cultured with TCR activator-Raji cells.

LAG3/IL-2 Reporter-Jurkat cells (BPS Bioscience #79813) were incubated with anti-MHC class II antibody (BioXCell #BE0306) and TCR activator-Raji cells (BPS Bioscience #60556). After incubation, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure IL-2 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.

Dose response of anti-MHC class II antibody in LAG3/IL-2 Reporter-Jurkat cells.

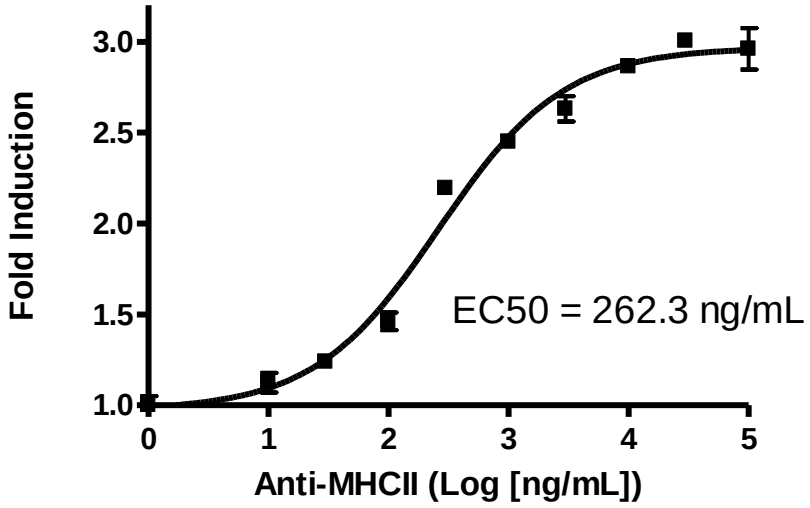

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Figure 3. Characterization of biological activity of anti-MHC class II antibody and anti-LAG3 antibody in cell-based assay using the LAG3/IL-2 Reporter-Jurkat cells or IL-2 reporter-Jurkat cells co-cultured with TCR activator-Raji cells.

LAG3/IL-2 Reporter-Jurkat cells (BPS Bioscience #79813) and IL-2 Reporter-Jurkat cells (BPS Bioscience #60481) were incubated with TCR activator-Raji cells (BPS Bioscience #60556) and 100 µg/mL anti-MHC class II antibody (BioXCell #BE0306) or 10 µg/ml anti-LAG3 neutralizing antibody (BPS Bioscience #71219). After incubation, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure IL-2 activity.

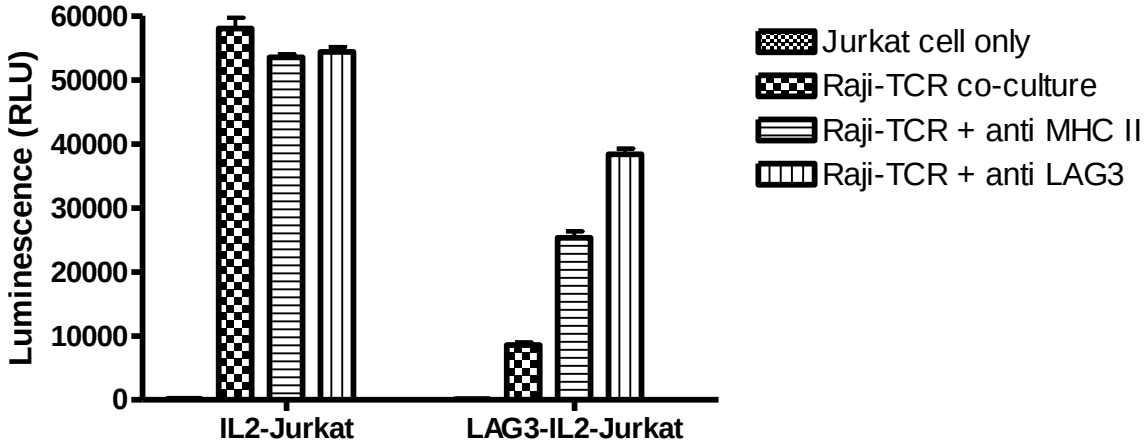
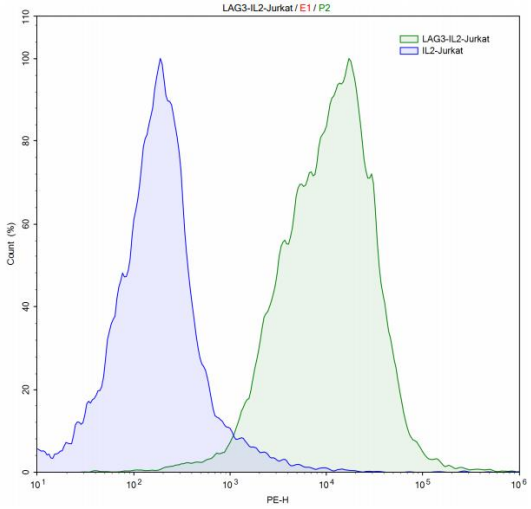


Figure 4 FACS analysis of cell surface expression of LAG3 in LAG3/IL-2 Reporter-Jurkat cells.

LAG3/IL-2 Reporter-Jurkat (BPS Bioscience #79813; green) or control IL-2 Reporter - Jurkat cells (BPS Bioscience #60481; blue) were stained with PE-labeled anti-LAG3 antibody (BPS Bioscience #71226) and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.



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Sequence

Human LAG3 sequence (accession number NM_002286)

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MWEAQFLGLLFLQPLWVAPVKPLQPGAEVPVVWAQEGAPAQLPCSPTIPLQDLSLLRRAGVTWQHQPDSGP
PAAAPGHPLAPGPHPAAPSSWGPRPRRYTVLSVGPGGLRSGRLPLQPRVQLDERGRQRGDFSLWLRPARRA
DAGEYRAAVHLRDRALSCRLRLRLGQASMTASPPGSLRASDWVILNCSFSRPDRPASVHWFRNRGQGRVPV
RESPHHHLAESFLFLPQVSPMDSGPWGCILTYRDGFNVSIMYNLTVLGLEPPTPLTVYAGAGSRVGLPCRL
PAGVGTRSFLTAKWTPPGGGPDLLVTGDNGDFTLRLEDVSQAQAGTYTCHIHLQEQQLNATVTLAIITVTP
KSFGSPGSLGKLLCEVTPVSGQERFVWSSLDTPSQRSFSGPWLEAQEAQLLSQPWQCQLYQGERLLGAAVY
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KIEELEQEPEPEPEPEPEPEPEPEPEQL
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Related Products

Product	Cat. #	Size
IL-2 Reporter – Jurkat cell line	60481	2 vials
TCR activator – Raji cell line	60556	2 vials
Anti-LAG3 neutralizing antibody	71219	100 µg
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
PE labeled anti-LAG3 antibody	71226-1	50 µg
PE labeled anti-LAG3 antibody	71226-2	100 µg
PD-1/IL-2 Reporter-Jurkat cell line	60535	2 vials
TCR Activator/PD-L1-CHO cell line	60536	2 vials
LAG3 (CD223), Fc fusion (Human)	71146	100 µg
LAG3 (CD223), Biotin-labeled (Human) HiP™	71147	50 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.