Description

Recombinant Jurkat T cell expressing firefly luciferase gene under the control of IL-2 response promotor 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 compound activity on LAG3 signaling in a cellular context.
- Characterize the biological activity of LAG3 and its interactions with ligands.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~2 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

Jurkat T Cell

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.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2J	BPS Bioscience #79812

Materials Required for Cellular Assay

Name	Ordering Information
TCR activator-Raji cells	BPS Bioscience #60556
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 or other luciferase reagents for measuring firefly luciferase activity	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2J.

Media Required for Cell Culture

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)

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).

Assay Medium: Thaw Medium 2 (BPS Bioscience #60184)

Cell Culture Protocol

Cell Thawing

1. 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**).
2. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 2 (**no Geneticin and Puromycin B**).
3. 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.
4. Cells should be split before they reach ~2 x 10^6 cells/ml.
5. At first passage, switch to Growth Medium 2J (**contains Geneticin and Puromycin B**).

Cell Passage

1. 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.

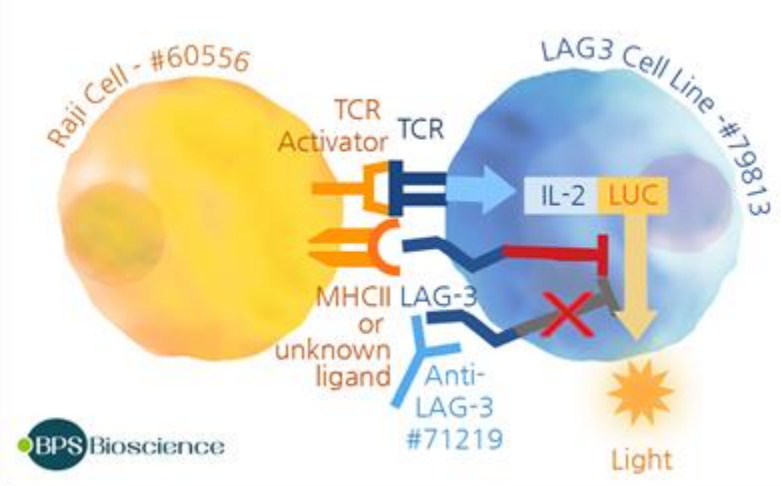
Note: Just after thawing, the cells may grow at a slower rate. It is recommended to split the cells at no less than 0.4 x 10^6 cells/ml at the beginning of culturing. After approximately two passages, the cell growth rate increases and the cells can be split to 0.2 x 10^6 cells/ml.

Cell Freezing

1. 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.
2. Dispense 1 ml of cell aliquots into cryogenic vials.
3. Place vials in an insulated container for slow cooling and store at -80°C overnight.
4. Transfer to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

Assay Principle



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.

1. Prepare serial dilutions of anti-LAG3 neutralizing antibody and other test compounds at 2x in assay medium and plate 50 µl/well into a white clear-bottom 96 well assay plate.

2. Harvest LAG3/IL-2 reporter Jurkat cells by centrifugation and resuspend in assay medium at 4.8 x 10^6 cells/ml. Add 25 µl of LAG3/IL-2 reporter cells/well to the 96 well plate with compound. Pre-incubate with test compounds at 37°C in a CO_2 incubator for 30 to 60 minutes.

3. Harvest TCR activator Raji cells by centrifugation and resuspend in assay medium at 0.4 x 10^6 cells/ml. Preincubate the cells at 37°C in a CO_2 incubator for approximately 30 minutes during the Jurkat + compound incubation.

4. Add 25 µl of TCR activator-Raji cells per well to the 96 well white clear-bottom assay plate containing compounds + LAG3/IL-2 Jurkat cells. Mix the plate gently. Final density of LAG-3/IL-2 Reporter-Jurkat cells is 120,000 cells/well and of TCR activator Raji is 10,000 cells/well. Final concentration of compound 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.

5. 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.

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.

Validation Data

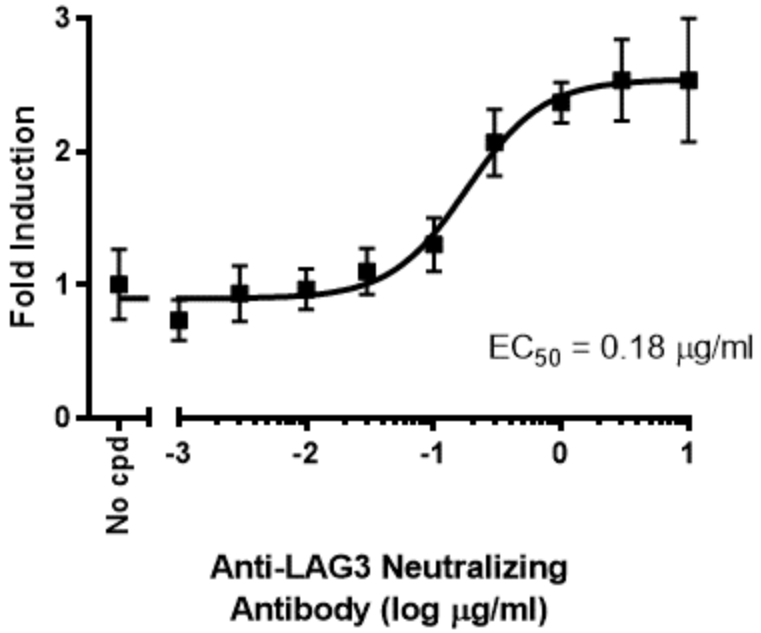


Figure 1. **Characterization of biological activity of anti-LAG3 neutralizing antibody in LAG3 cell-based assay using the LAG3/IL-2 Reporter-Jurkat cells co-cultured with TCR activator-Raji cells.**
LAG3/IL-2 Reporter-Jurkat cells (BPS Bioscience #79813) were incubated with anti-LAG3 neutralizing antibody (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.

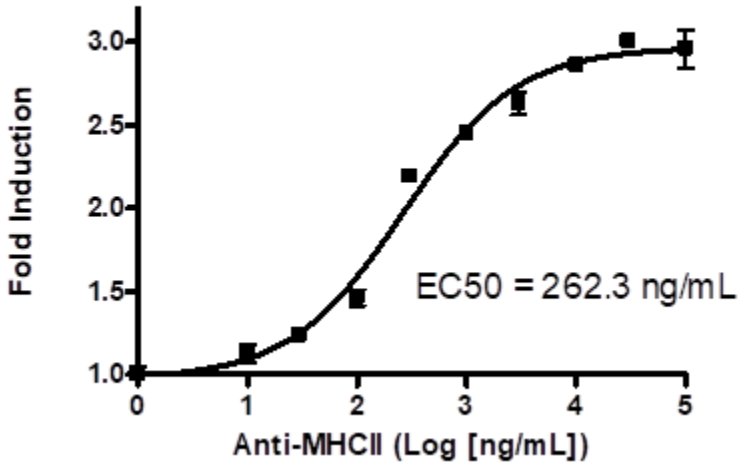


Figure 2. **Characterization of biological activity of anti-MHC class II antibody in LAG3 cell-based assay using the LAG3/IL-2 Reporter-Jurkat cells co-cultured with TCR activator-Raji cells.**
LAG3/IL-2 Reporter-Jurkat cells (BPS Bioscience #79813) were incubated with anti-MHC class II antibody (BioXCell #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.

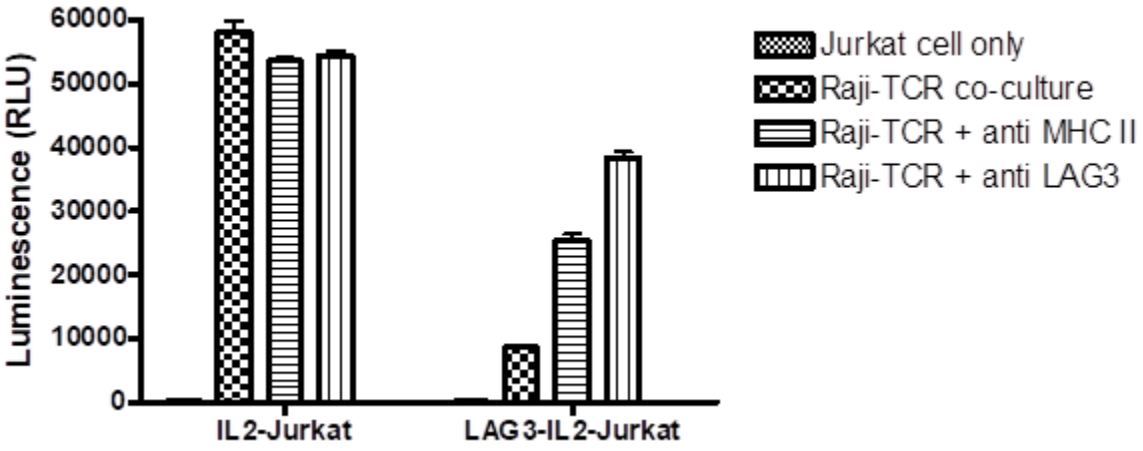


Figure 3. **Characterization of biological activity of anti-MHC class II antibody and anti-LAG3 antibody in cell-based assay using the LAG3/IL-2 Reporter-Jurkat cells or IL-2 reporter-Jurkat cells co-cultured with TCR activator-Raji cells.**

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™TM Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure IL-2 activity.

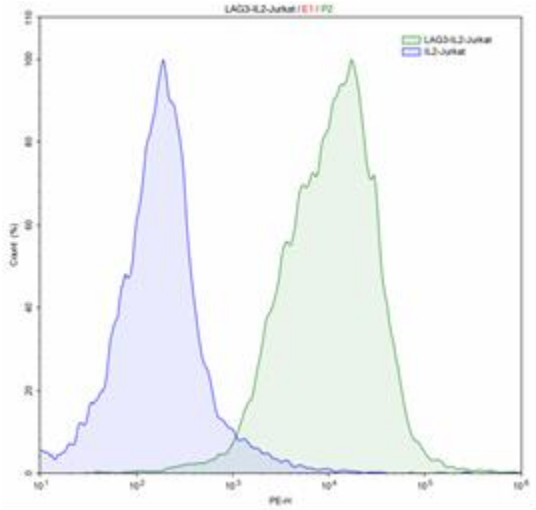


Figure 4: **FACS analysis of cell surface expression of LAG3 in LAG3/IL-2 Reporter-Jurkat cells.**

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.

Sequence

Human LAG3 sequence (GenBank Accession Number NM_002286)

MWEAQFLGLLFLQPLWVAPVKPLQPGAEVPVVWAQEGAPAQLPCSPTIPLQDLSLLRRAGVTWQHQPDSGPPAAAPGHPLAP
GPHPAAPSSWGPRPRRYTVLSVGPGGLRSGRLPLQPRVQLDERGRQRGDFSLWLRPARRADAGEYRAAVHLRDRALSCRLRLRL
GQASMTASPPGSLRASDWVILNCSFSRPDRPASVHWFRNRGQGRVPVRESPHHHLAESFLFLPQVSPMDSGPWGCILTYRDGF
NVSIMYNLTVLGLEPPTPLTVYAGAGSRVGLPCRLPAGVGTRSFLTAKWTPPGGGPDLLVTGDNGDFTLRLEDVSQAQAGTYTC
HIHLQEQQLNATVTLAIITVTPKSFGSPGSLGKLLCEVTPVSGQERFVWSSLDTPSQRSFSGPWLEAQEAQLLSQPWQCQLYQGE
RLLGAAVYFTELSSPGAQRSGRAPGALPAGHLLLFLILGVLSLLLLVTGAFGFHLWRRQWRPRRFSALEQGIHPPQAQSKIEELEQE
PEPEPEPEPEPEPEPEPEQL

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*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™^{T} Luciferase Assay System	60690	Multiple Sizes
Anti-LAG3 Antibody, PE-Labeled	71226-1	50 µg
Anti-LAG3 Antibody, PE-Labeled	71226-2	100 µg
PD-1/NFAT-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