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Data Sheet

LILRB4 NFAT-Luciferase Reporter Jurkat Recombinant Cell Line
Catalog #79750

Product Description
Recombinant Jurkat cells constitutively expressing human LILRB4 (Leukocyte Immunoglobulin-Like Receptor Subfamily B Member 4, GenBank Accession #NM_001278426) and the firefly luciferase gene under the control of NFAT response elements.

Background
Leukocyte Immunoglobulin-Like Receptor Subfamily B Member 4 (LILRB4) contains multiple Immunoreceptor Tyrosine-based Inhibitory Motifs (ITIMs). LILRB4 expression on Acute Myeloid Leukemia (AML) cells promotes tumor cell infiltration and suppresses T cell activity. Because LILRB4 is highly expressed on AML cells but not on normal hematopoietic stem cells, LILRB4 has been identified as a promising therapeutic target for CAR-T cells.

Applications
- Screen for novel ligands of LILRB4

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

Storage
Immediately upon receipt, store in liquid nitrogen.

Culture conditions

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

Growth Medium 2F (BPS Bioscience, #79669): Thaw Medium 2 (BPS Bioscience, #60184) plus 1 mg/ml of Geneticin (Thermo Fisher, #11811031), 0.5 µg/ml Puromycin Dihydrochloride (Thermo Fisher, #A1113803) to ensure recombinant expression.

Cells should be grown at 37˚C with 5% CO_2 using Growth Medium 2F to ensure recombinant expression is maintained.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37˚C water-bath, and then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 **without Geneticin or Puromycin.** Then spin the cells down, remove the supernatant, and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2 **without Geneticin or Puromycin.**

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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 Thaw Medium 2 **without Geneticin or Puromycin**. At first passage, switch to Growth Medium 2F **containing Geneticin and Puromycin**. Cells should be split before they reach 2 x 10^6 cells/ml.

Functional Validation and Assay Performance
Expression of human LILRB4 in the NFAT-Luciferase Jurkat cells was confirmed by FACS.

The functionality of the cell line was validated using an anti-CD3 Agonist Antibody (BPS Bioscience, #71274), and compared with the parental NFAT-Luciferase Jurkat cells (BPS Bioscience, #60621). Treatment with the anti-CD3 Agonist Antibody induces NFAT Luciferase activity, which is partially inhibited by LILRB4 expression.

Figure 1. FACS analysis of LILRB4 NFAT-Luciferase Jurkat cells.
FACS analysis was performed using a PE anti-human LILRB4 antibody from BioLegend (#333008). Parental NFAT-Luciferase Jurkat cells are shown in green, and LILRB4 NFAT-Luciferase Jurkat cells are shown in blue.

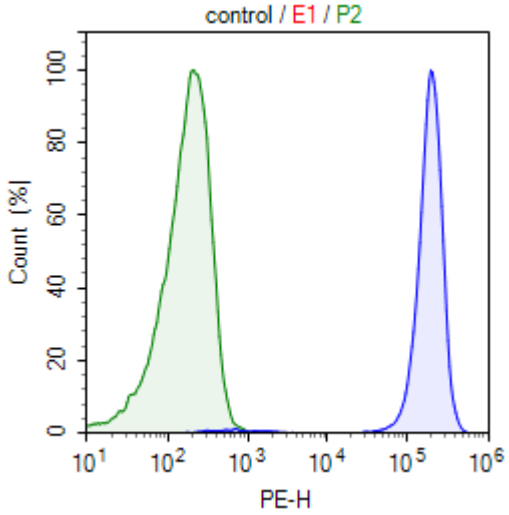

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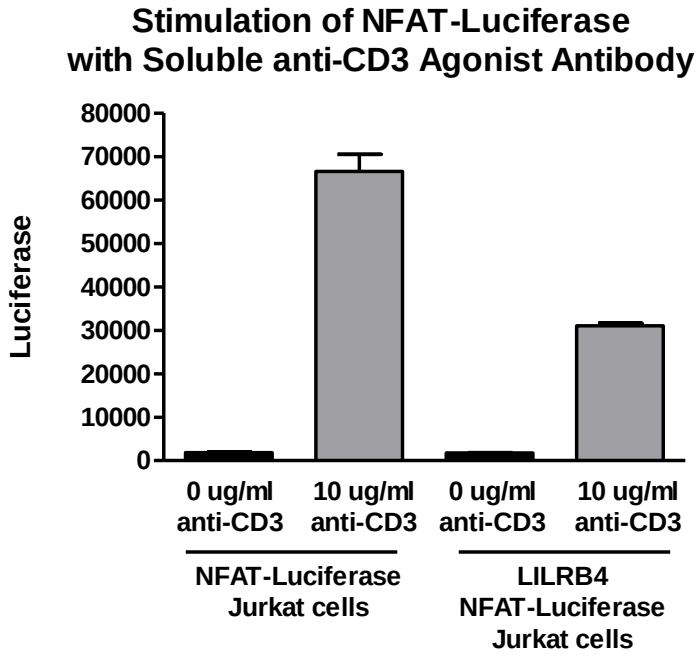
Figure 2. Stimulation of NFAT-Luciferase activity with soluble anti-CD3 Agonist Antibody. Parental NFAT-Luciferase Jurkat cells or LILRB4 NFAT-Luciferase Jurkat cells were plated in triplicate at 15,000 cells/well into a clear-bottom 96-well plate. Cells were then incubated with either 0 or 10 µg/ml anti-CD3 Agonist Antibody for 5 hours at 37˚C. After incubation, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to the cells to measure NFAT activity.



Materials Required for Cell Culture

- Thaw Medium 2 (BPS Bioscience #60184)
- Growth Medium 2F (BPS Bioscience #79669)

Materials Required for Cellular Assay

- Assay Medium (Thaw Medium 2 (BPS Bioscience, #60184))
- Thaw Medium 3 (BPS Bioscience, #60186):
  - Ham's F-12 medium (Hyclone # SH30526.01) supplemented with 10% FBS (Thermo Fisher, Cat. #26140079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).
- TCR activator CHO cells (BPS Bioscience, #60539)
- Anti-CD3 Agonist Antibody (BPS Bioscience, #71274)
- NFAT-Luciferase Jurkat Recombinant Cell Line (BPS Bioscience, #60621)
- ANGPTL3 (BPS Bioscience, #91009)
- ANGPTL7 (RND Systems, #914-AN-025)
- 96-well tissue culture-treated white clear-bottom assay plate

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- One-Step luciferase assay system (BPS Bioscience, #60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

Assay Protocol for Testing Soluble Ligands

1. Harvest TCR activator CHO cells (BPS Bioscience, #60539) from culture and seed cells at a density of 15,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of Thaw Medium 3. Leave a couple of wells empty for use as a cell-free control. Incubate the cells at 37˚C in a CO_2 incubator overnight.

2. The next day, harvest the LILRB4 NFAT-Luciferase Jurkat cells by centrifugation and resuspend in Assay Medium (Thaw Medium 2, **no Geneticin or Puromycin**). Dilute the cells to 6 x 10^5 cells/ml in the Assay Medium.

   Prepare dilutions of the Test Ligand (e.g. ANGPTL3 or ANGPTL7; 25 µg/ml) in 25 µl of the Assay Medium, and mix with 25 µl of the LILRB4 NFAT-Luciferase Jurkat cells. Incubate the Test Ligand with the LILRB4 NFAT-Luciferase Jurkat cells for 30 minutes at room temperature. After incubation, remove the Thaw Medium 3 from the TCR activator CHO cells and add 50 µl of the LILRB4 NFAT-Luciferase Jurkat cells / Ligand mixture to the TCR activator CHO cells.

   The final cell density of the LILRB4 NFAT-Luciferase Jurkat cells is 15,000 cells per well. Set up each treatment in at least triplicate. Add 50 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.

3. The next day, after a 24-hour incubation, measure the luciferase activity using the ONE-Step Luciferase Assay System: Prepare the ONE-Step reagent as recommended in the protocol. Add 50 µl of ONE-Step Luciferase Reagent per well and rock gently at room temperature for ~15 minutes. Measure the luminescence using a luminometer.
   If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

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

   The fold induction of NFAT luciferase reporter expression = background-subtracted luminescence of treated well / average background-subtracted luminescence of untreated control wells.

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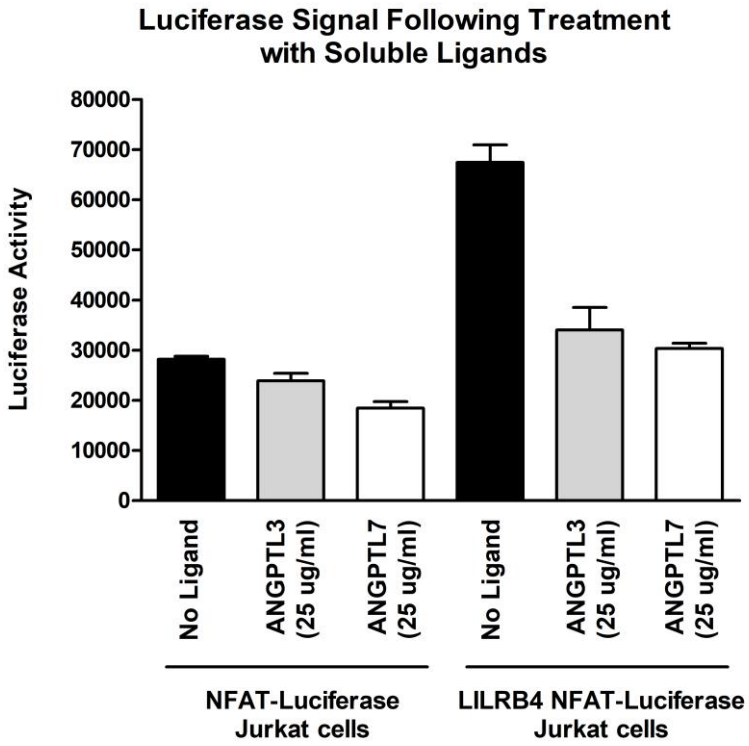
Figure 3. Inhibition of LILRB4 NFAT-Luciferase activity using soluble ligands.
TCR activator CHO cells were seeded in a 96-well plate. The next day, NFAT-Luciferase Jurkat cells or LILRB4 NFAT-Luciferase Jurkat cells were pre-incubated with either ANGPTL3 (BPS Bioscience, #91009) or ANGPTL7 (RND Systems, #914-AN-025) for 30 minutes, and then the Jurkat cells / ligand mixture were added to the TCR activator CHO cells and incubated for 24 hours. After incubation, ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to the cells to measure NFAT-Luciferase activity.



Sequence
Human LILRB4 sequence (accession number NM_001278426):

MIPTFTALLCLGLSLGPRTHMQAGPLPKPTLWAEPGSVISWGNSVTIWCQGTLEAREYRLDKEESPAPWDRQNPLEP
KNKARFSIPSMTEDYAGRYRCYYRSPVGWSQPSDPLELVMTGAYSKPTLSALPSPLVTSGKSVTLLCQSRSPMDTFL
LIKERAAHPLLHLRSEHGAQQHQAEFPMSPVTSVHGGTYRCFSSHGFSHYLLSHPSDPLELIVSGSLEGPRPSPTRS
VSTAAGPEDQPLMPTGSVPHSGLRRHWEVLIGVLVVSILLLSLLLFLLLQHWRQGKHRTLAQRQADFQRPPGAAEPE
PKDGGLQRRSSPAADVQGENFCAAVKNTQPEDGVEMDTRQSPHDEDPQAVTYAKVKHSRPRREMASPPSPLSGEFLD
TKDRQAEEDRQMDTEAAASEAPQDVTYARLHSFTLRQKATEPPPSQEGASPAEPSVYATLAIH

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References:

1. LILRB4 signaling in leukemia cells mediates T cell suppression and tumor infiltration. Deng M, *et al. Nature*. 2018. **562(7728):**605-609.

2. A novel anti-LILRB4 CAR-T cell for the treatment of monocytic AML. John S, *et al*. *Mol Ther.* 2018. **26(10):**2487-2495.

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Related Products

Product	Cat. #	Size
LILRA1, Avi-His Tag	100245	100 µg
LILRB2, Avi-His Tag	100234	100 µg
LILRB4, Avi-His Tag	100236	100 µg
LILRB1, Fc fusion, Biotin-labeled	79474	100 µg
LILRB1, Fc fusion	79473	100 µg
Thaw Medium 2	60184	100 ml
Growth Medium 2F	79669	500 ml
Thaw Medium 3	60186	100 ml
TCR activator CHO cells	60539	2 vials
Anti-CD3 Agonist Antibody	71274-1	50 µg
Anti-CD3 Agonist Antibody	71274-2	100 µg
NFAT-Luciferase Jurkat Cell Line	60621	2 vials
ANGPTL3	91009-1	10 µg
ANGPTL3	91009-2	50 µg
ANGPT1	91002	50 µg
ANGPT2	91003	50 µg