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Produktinformation

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

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The IL-33 Responsive Luciferase Reporter Jurkat Cell Line is a Jurkat cell line engineered to express both human IL33Ra (IL-33 receptor alpha) (NM_016232.5) and human IL1RAP (IL-1 Receptor Accessory Protein) (NM_002182.4) separated by a self-cleaving P2A peptide. The construct was delivered by lentiviral transduction of NF-κB Luciferase Reporter Jurkat Cells (#60651), which express a firefly luciferase reporter driven by NF-κB (nuclear factor Kappa B) response elements located upstream of the minimal TATA promoter. After activation by IL-33, the endogenous transcription factor NF-κB binds to the response elements, inducing transcription of the luciferase reporter gene.

This cell line has been validated to respond to IL-33.

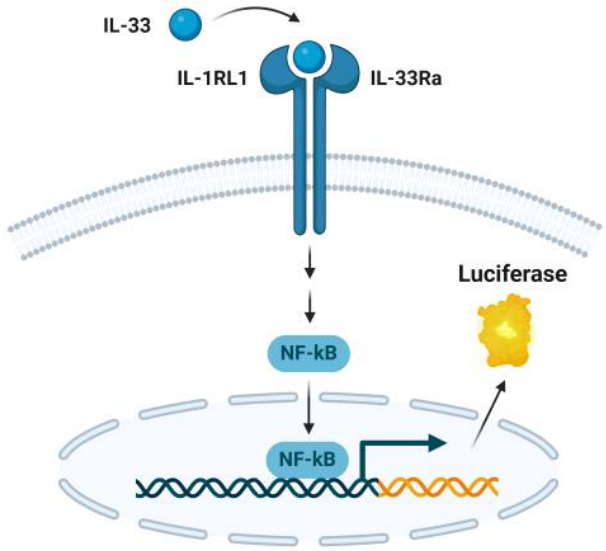

Figure 1: Illustration of the mechanism of IL-33 Luciferase Reporter Jurkat Cell Line.
IL-33 binds to its cognate receptor to activate downstream signal pathways that converge on transcription factor NF-κB, which translocates into the nucleus where it can activate the transcription of the Firefly luciferase reporter driven by NF-κB response elements present in the promoter.

Background

Interleukin-33 (IL-33) is a tissue-derived alarmin cytokine belonging to the Interleukin-1 (IL-1) family that regulates tissue homeostasis and inflammation and plays a key role in allergic airway diseases. IL-33 is produced constitutively by a variety of cell types, including fibroblasts, endothelial and epithelial cells, where it is stored in the cell nucleus and subsequently released in response to cell necrosis, cellular damage, stress, and tissue injury. After release, IL-33 primarily drives type 2 immune responses by activating T_H2 (T helper 2) cells, mast cells, and innate lymphoid cells (ILC2s). IL-33 signals through ST2 (receptor serum-stimulated 2), also known as IL-1RL1, and its co-receptor IL-1RAcP, and activates downstream signaling pathways including NF-κB and MAPK (mitogen-activated protein kinase), leading to the production and release of pro-inflammatory cytokines. IL-33 is a promising therapeutic target for inflammatory diseases including asthma and COPD (chronic obstructive pulmonary disease), with anti-IL-33 neutralizing antibodies (including Tozorakimab) currently undergoing clinical trials.

Application

- Screen IL-33 antibodies.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ≥1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience, #79796)

Parental Cell Line

Jurkat (clone E6-1), human T lymphoblast, suspension.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2A	BPS Bioscience #60190

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Human Interleukin-33 Recombinant	BPS Bioscience #90191-A
Anti-IL33 Neutralizing Antibody	BPS Bioscience #102416
Tozorakimab	BPS Bioscience #82868
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
White, clear-bottom cell culture plate, 96-well	Corning #3610
White, Tissue Culture treated plate, 384-well	PerkinElmer #6007680
Luminometer	

Storage Conditions

Cells are shipped in 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, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Growth Medium 2A (BPS Bioscience #60190):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin, and 200 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: Jurkat cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 2.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2.

3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2A.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml in Growth Medium 2A. The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

2. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

3. Transfer the vials to liquid nitrogen the next day for long term storage.

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

Functional Validation

- The following assays are designed for 96-well (protocol A) and 384-well format (protocol B). To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The experiments should be performed in triplicate.
- Assay A and B should include "Cell-Free Control", "Unstimulated Control" and "Stimulated" conditions.
- Assay C should include "Stimulated, No Compound", "Unstimulated, No Compound", "Background Luminescence" and "Test Compound" conditions.

Assay Medium: *Thaw Medium 2*

A. 96-Well Assay Format: Dose-response of IL-33 Responsive Luciferase Reporter Jurkat Cell Line to recombinant human IL-33.

1. Seed IL-33 Responsive Luciferase Reporter Jurkat cells into a white clear-bottom 96-well microplate at a density of 35,000 - 40,000 cells per well in 90 µl of Assay Medium. Leave a few empty wells to determine the background luminescence ("Cell-Free Control").

2. Incubate cells at 37°C in a CO_2 incubator overnight.

3. Prepare a serial dilution of recombinant human IL-33 at concentrations 10-fold higher than the desired final concentrations in Assay Medium (10 µl/well).

4. Add 10 µl of each dilution to the "Stimulated" wells.

5. Add 10 µl of Assay Medium to the "Unstimulated Control" (negative control) wells.

6. Add 100 µl of Assay Medium to the "Cell-Free Control" wells (for determining background luminescence).
7. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.
8. Add 100 µl of the ONE-Step™ Luciferase reagent to each well.
9. Rock gently at Room Temperature (RT) for ~15 minutes.
10. Measure luminescence using a luminometer.

Data Analysis

Subtract the average background luminescence (cell-free wells) from the luminescence reading of all wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ Stimulated\ Wells\ -\ avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells\ -\ avg.background}$$

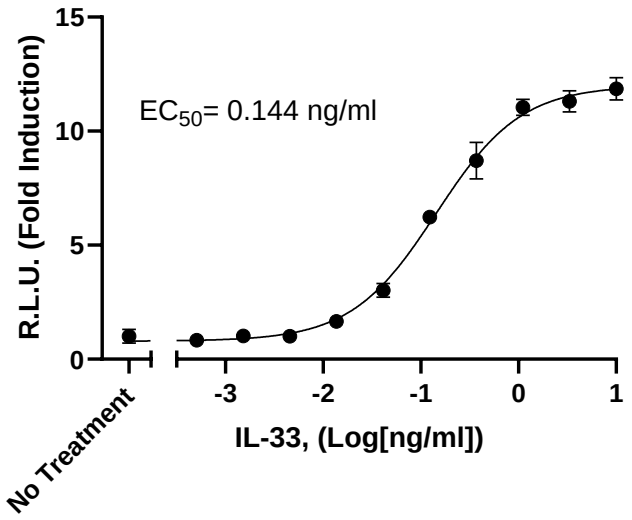

Figure 2. Dose response curve of IL-33 Responsive Luciferase Reporter Jurkat Cell Line to recombinant human IL-33 in a 96-well assay format.
Cells were treated with increasing concentrations of IL-33 in a 96-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

B. 384-Well Assay Format: Dose-response of IL-33 Responsive Luciferase Reporter Jurkat Cell Line to recombinant human IL-33.

1. Seed IL-33 Responsive Luciferase Reporter Jurkat cells into a white 384-well plate at a density of 10,000 cells per well in 20 µl of Assay Medium. Leave a few empty wells to determine the background luminescence ("Cell-Free Control").
2. Incubate cells at 37°C in a CO_2 incubator overnight.
3. Prepare a serial dilution of IL-33 at 5X the final testing concentration (5 µl/well).
4. Add 5 µl of IL-33 to the "Stimulated Cells" wells.
5. Add 5 µl of Assay Medium to the "Unstimulated Control" (negative control) wells.
6. Add 25 µl of Assay medium to the "Cell Free Control" wells.
7. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.
8. Add 25 µl of the ONE-Step™ Luciferase reagent to each well.
9. Rock gently at RT for ~15 minutes.
10. Measure luminescence using a luminometer.

Data Analysis

Subtract the average background luminescence (cell-free wells) from the luminescence reading of all wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ Stimulated\ Wells\ -\ avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells\ -\ avg.background}$$

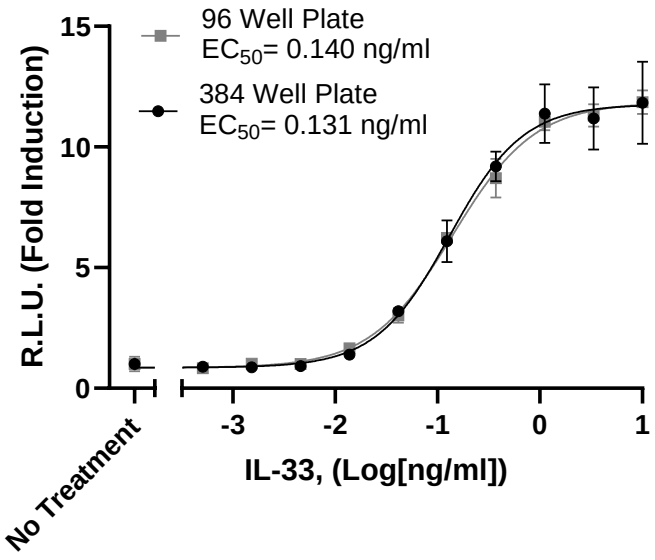

Figure 3. Dose-response curve of IL-33 Responsive Luciferase Reporter Jurkat Cell Line to recombinant human IL-33 in 96 and 384-well format.
Cells were treated with increasing concentrations of IL-33 in a 384-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control.

C. Inhibition of IL-33 stimulation by anti-IL-33 antibodies

- This experiment measures the effect of neutralizing antibodies against IL-33 stimulation.

1. Plate IL-33 Responsive Luciferase Reporter Jurkat cells at a density of 40,000 cells/well in 80 µl of Assay Medium into a white, clear-bottom white 96-well plate. Leave a few empty wells as "Background Luminescence" control.

2. Incubate the plate at 37°C with 5% CO_2 overnight.

3. Prepare a solution of human IL-33 at 10x the final concentration in Assay Medium (10 µl/well).

4. Prepare a 3-fold serial dilution of anti-IL-33 antibody at 10x the desired final concentration in Assay Medium (10 µl/well).

5. Combine equal volumes of IL-33 and anti-IL-33 antibody prepared in step 3 and 4, mix, and add 20 µl of antibody mixture to "Test Compound" wells.

6. Add 10 µl of human IL-33 and 10 µl of Assay Medium to the "Stimulated, No Compound" wells.

7. Add 20 µl of Assay Medium to "Unstimulated, No Compound" wells.
8. Add 100 µl of Assay Medium to "Background Luminescence" control wells.
9. Incubate the plate at 37°C with 5% CO_2 for 5-6 hours.
10. Add 100 µl of the ONE-Step™ Luciferase reagent to each well.
11. Rock gently at RT for ~15 minutes.
12. Measure luminescence using a luminometer.
13. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells. The Percent Luminescence of IL-33 luciferase reporter expression is the background-subtracted luminescence of "Test Compound" cells divided by the background-subtracted luminescence of the "Stimulated, No Compound" treated wells.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ compound\ treated\ cells - background}{luminescence\ of\ stimulated, no\ compound\ treated\ cells - background}\right) x100$$

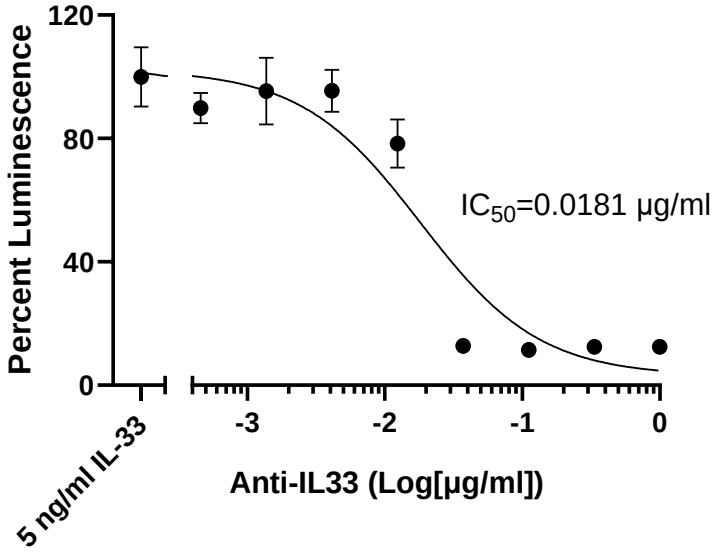

Figure 4: Dose-dependent response of IL-33 Responsive Luciferase Reporter Jurkat Cell Line to Anti-IL-33 Neutralizing Antibody.
Cells were incubated with increasing concentrations of Anti-IL-33 Neutralizing Antibody (#102416) and 5 ng/ml of IL-33 for 6 hours. Luciferase activity was measured using One-Step™ Luciferase

Assay System. The results are shown as percentage of NF-κB luciferase reporter activity compared to the activity of cells without antagonist (set at 100%).

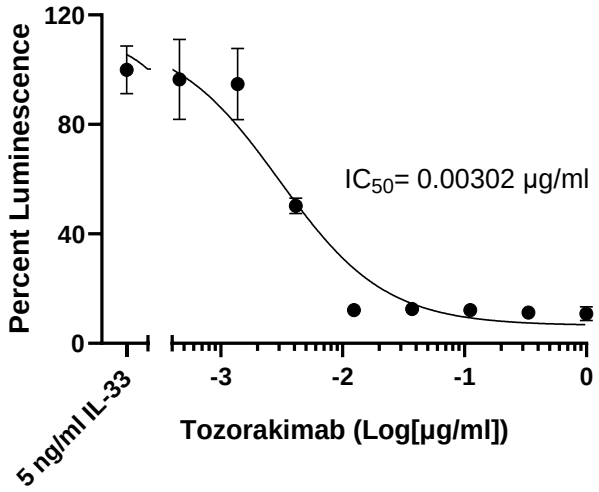

Figure 5. Dose-dependent response of IL-33 Responsive Luciferase Reporter Jurkat Cell Line to Tozorakimab.
Cells were incubated with increasing concentrations of Tozorakimab and 5 ng/ml of IL-33 for 6 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. The results are shown as percentage of NF-κB luciferase reporter activity compared to the activity of cells without antagonist (set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Cayrol C., *et al.,* 2018 *Immunol Rev.* 281(1):154-168.
Drake L., *et al.,* 2017 *Immunol Rev.* 278(1):173-184.
England E., *et al.,* 2023 *Scientific reports.* 13(1):9825.
Zhou Y., *et al.,* 2023 *J Transl Med.* 21(1):902.

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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*
NF-κB Luciferase Reporter Jurkat Cell Line	60651	2 vials
IL-31 Responsive Luciferase Reporter HEK293 Cell Line	82799	2 vials
IL-23 Responsive STAT3 Luciferase Reporter HEK293 Cell Line	82591	2 vials
IL1RL1: IL33 [Biotinylated] Inhibitor Screening Chemiluminescent Assay Kit	82856	96 reactions

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