Description

TNF-like ligand 1A (TL1A)-Responsive Luciferase Reporter Jurkat Cell Line is a TL1A-responsive DR3/NF-kB luciferase reporter Jurkat cell line expressing firefly luciferase under the control of an NF-kB response element, and with stable expression of human DR3 (death receptor 3; TNFRSF25; NM_003790.3). Expression of the firefly luciferase reporter is driven by NF-kB response elements located upstream of the minimal TATA promoter. Activation of the NF-kB signaling pathway by the DR3 ligand TL1A (TNF-like Protein 1A; TNFSF15) can be monitored by measuring luciferase activity.

This cell line has been validated to be responsive to TL1A and TNF-α stimulation. TL1A stimulation was blocked by the neutralizing anti-TL1A antibody (#101729) and DcR3 (decoy receptor 3).

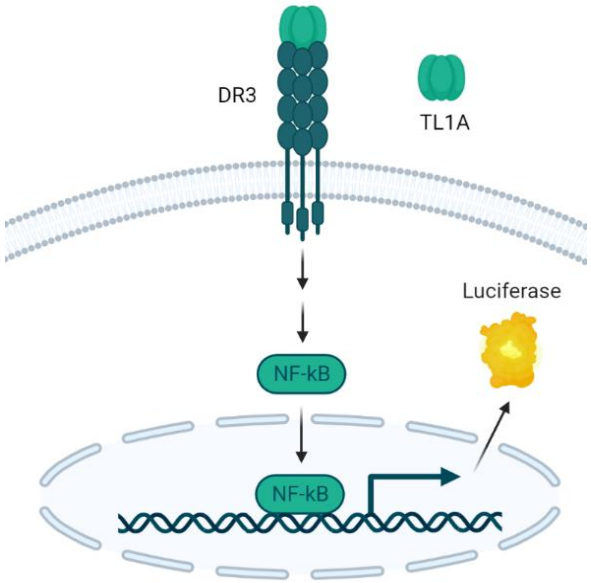

Figure 1: Schematic representation of the TL1A response in TL1A Responsive Luciferase Reporter Jurkat Cell Line.

Background

TNF-like ligand 1A (TL1A, also known as Vascular endothelial growth inhibitor, VEGI or TNFSF15) is an anti-angiogenic cytokine. It is an important mediator of inflammation, participating in innate and adaptive immune homeostasis through binding to its receptor, DR3 (death receptor 3), activating downstream signaling. Numerous studies showed that soluble TL1A can be detected in the serum of patients with T-cell mediated autoimmune diseases like rheumatoid arthritis, psoriatic arthritis, and inflammatory bowel disease. In addition, recent clinical studies suggested that anti-TL1A antibody treatment is a promising therapeutic approach in inflammatory and auto-immune disorders.

Application

- Characterize soluble TL1A activity.
- Screen anti-TL1A antibodies in a cell-based assay format.

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)

Host Cell
Jurkat cells (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.

Materials 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
TL1A, His-Tag, Avi-Tag Recombinant	BPS Bioscience #101880
Anti-TL1A Antibody	BPS Bioscience #101729
Recombinant Human TNF-alpha Protein	R&D Systems #210-TA
Human Recombinant DcR3/TNFRSF6B Fc chimera	R&D Systems #142-DC
NF-κB Luciferase Reporter Jurkat Cell Line	BPS Bioscience #60651
Growth Medium 2A	BPS Bioscience #60190
Assay Medium: Thaw Medium 2	BPS Bioscience #60184
96-well tissue culture treated, white, clear-bottom assay plate	
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
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(s) 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):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2A (BPS Bioscience #60190):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 200 µg/ml of Hygromycin and 1 mg/ml Geneticin.

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

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

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

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 2 to the conical tube containing the cells. Thaw Medium 2 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. 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.

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

6. Check the cell viability after 24 hours in culture. For a T25 flask, add 3-4 ml of Thaw Medium 2, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. 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 used should maintain the cells between 0.2 x 10^6 to 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.

Validation

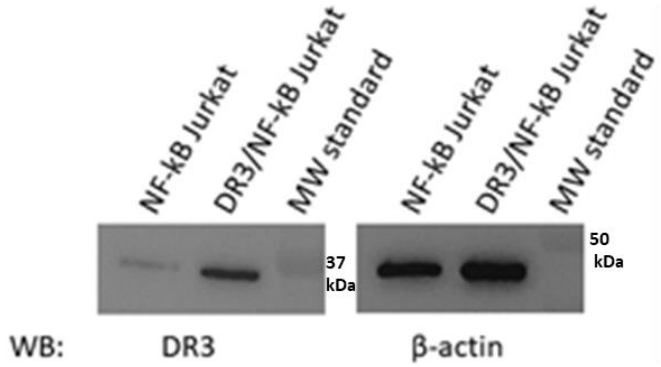

Figure 2: DR3 protein expression in parental NF-kB Luciferase Reporter Jurkat Cell Line (NF-κB Jurkat) and TL1A-Responsive Luciferase Reporter Jurkat Cell Line (DR3/NF-kB Jurkat).
Cells were lysed and human DR3 expression levels were analyzed by SDS-PAGE electrophoresis followed by Western Blotting using DR3 Recombinant Monoclonal Antibody (11H6L9) (ThermoFisher #702277). Actin was used as loading control and was detected with β-Actin (13E5) Rabbit mAB (Cell Signaling #4970).

Functional Validation

- The following assays are designed for a 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.
- The assay should be performed in triplicate.
- We recommend using NF-κB Luciferase Reporter Jurkat Cell Line as control.

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

A. Agonist evaluation

- This experiment assesses the effect of an agonist on reporter activation.
- The assay should include "Stimulated", "Background Luminescence" (no cells) and "Unstimulated" (negative control, no agonist) conditions.

1. Grow cells to ~1.5 x 10^6 cells/ml the day before the experiment.

2. Dilute cells with fresh Growth Medium 2A to bring the cell density to ~0.3 to 0.5 x 10^6 cells/ml.

3. Incubate the cells at 37° with 5% CO_2 overnight.

4. The next day, harvest the cells by centrifugation at 300 *x g* for 5 minutes and resuspend in Thaw Medium 2 at a density of 0.3 x 10^6 cells/ml.

5. Dispense 90 µl/well of cell suspension (~30,000 cells/well) into a white clear bottom 96-well plate. Keep a few wells without cells ("Background Luminescence" control wells).

6. Prepare a serial dilution of agonists, such as TL1A, in Thaw medium 2 at concentrations 10-fold higher than the desired final concentrations.

7. Add 10 µl of the diluted agonist to each well "Stimulated" well.

8. Add 10 µl of Thaw Medium 2 in the "Unstimulated" negative control wells.

9. Add 100 ml of Thaw Medium 2 to "Background Luminescence" (no cells) wells.

10. Incubate the cells at 37° with 5% CO_2 for ~5 hours.

11. After ~5 hours, add 100 µl of the ONE-Step™ Luciferase reagent per well.

12. Rock at room temperature for ~15 minutes.

13. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all conditions. The fold induction of NF-κB luciferase reporter expression is the background-subtracted luminescence of stimulated cells divided by the background-subtracted luminescence of unstimulated control cells.

$$Fold\ induction = \frac{(luminescence\ of\ stimulated\ cells - background)}{(luminescence\ of\ unstimulated\ cells - background)}$$

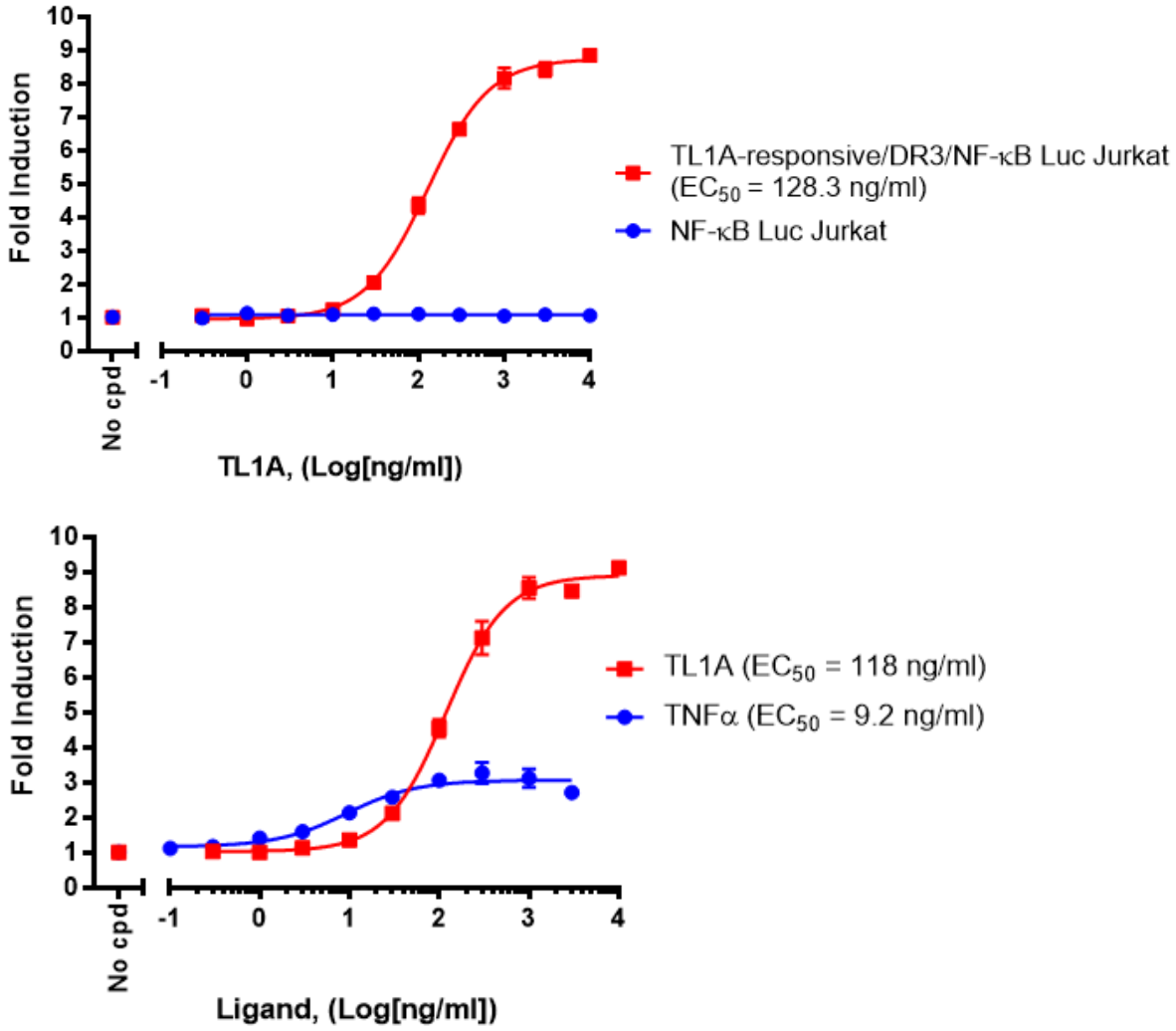

Figure 3. Dose-response to TL1A and TNFα in the TL1A Responsive Luciferase Reporter Jurkat Cell Line. Top panel: TL1A Responsive Luciferase Reporter Jurkat and NF-κB Luciferase Reporter Jurkat cells were treated with increasing concentrations of TL1A for 5 hours prior to performing the ONE-Step™ Luciferase assay. The results are shown as fold induction of luciferase reporter expression, determined by comparing luciferase activity of stimulated cells against the activity of the control wells without treatment. Jurkat cells that do not express DR3 (NF-κB Luciferase Reporter Jurkat Cell Line, #60651) do not show stimulation by TL1A.
Bottom panel: TL1A Responsive Luciferase Reporter Jurkat cells were treated with increasing concentrations of TL1A or TNFα for 5 hours prior to performing the ONE-Step™ Luciferase assay. The results are shown as fold induction of luciferase reporter expression, determined by comparing luciferase activity of stimulated cells against the activity of the control wells without treatment.

B. Inhibition of TL1A stimulation by Anti-TL1A Neutralizing Antibody and DcR3 Fc chimera

- This experiment assesses the effect of a neutralizing anti-TL1A antibody and an DcR3 Fc chimera against stimulation by TL1A.
- The assay should have "Background Luminescence" (no cells), "Unstimulated Control" (no TL1A, to determine the uninduced reporter activity and the range of the assay), and "Untreated Positive Control" (TL1A without neutralizing antibody or DcR3 chimera) and "Treated cells" conditions.

1. Grow cells to ~1.5 x 10^6 cells/ml the day before the experiment.

2. Dilute the cells with fresh Growth Medium 2A to bring the cell density to ~0.3 to 0.5 x 10^6 cells/ml.

3. Incubate the cells at 37° with 5% CO_2 overnight.

4. The next day, harvest the cells by centrifugation at 300 *x g* for 5 minutes and resuspend in Thaw Medium 2 at a density of 0.6 x 10^6 cells/ml.

5. Dispense 50 µl/well of cell suspension (~30,000 cells/well) into a white clear bottom 96-well plate. Keep wells without cells ("Background Luminescence" control wells).

6. Dilute TL1A to 4 µg/ml with Thaw Medium 2 (100 µl/well).

7. Prepare a serial dilution of anti-TL1A antibody or DcR3 Fc chimera in Thaw Medium 2 at concentrations 4-fold higher than the desired final concentrations (100 µl/well).

8. Prepare a separate 96 well plate for pre-incubations (**Pre-incubation plate**):

   8.1 Pre-incubate diluted anti-TL1A antibody or DcR3 Fc chimera with the diluted TL1A for 1 hour (100 µl of anti-TL1A + 100 µl of TL1A). This is the **TL1A/Antibody Mix**.

   8.2 Add 100 µl of diluted TL1A and 100 µl of Thaw Medium 2 to the **TL1A Only Mix** (for "Untreated Positive Control" wells).

9. Transfer 50 µl of **TL1A/Antibody Mix** from the Pre-incubation plate to the "**Treated Cells**" wells in the experimental plate.

10. Transfer 50 µl of **TL1A Only Mix** from the Pre-incubation plate to the "**Untreated Positive Control**" wells in the experimental plate.

11. Add 50 µl of Thaw Medium 2 to the "**Unstimulated Control**" wells (uninduced level of NF-kB reporter activity).

12. Add 100 µl of Thaw Medium 2 to "Background luminescence" (no cells).

13. Incubate the plate at 37°C with 5% CO_2 for ~5 hours.

14. Add 100 µl per well of the ONE-Step™ Luciferase reagent.

15. Rock at room temperature for ~15 minutes.

16. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the average luminescence reading of all conditions. The percent luminescence is the background-subtracted luminescence of antibody-treated cells divided by the background-subtracted luminescence of untreated control cells x 100.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ antibody\ treated\ cells - background}{luminescence\ of\ untreated\ cells - background}\right) x100$$

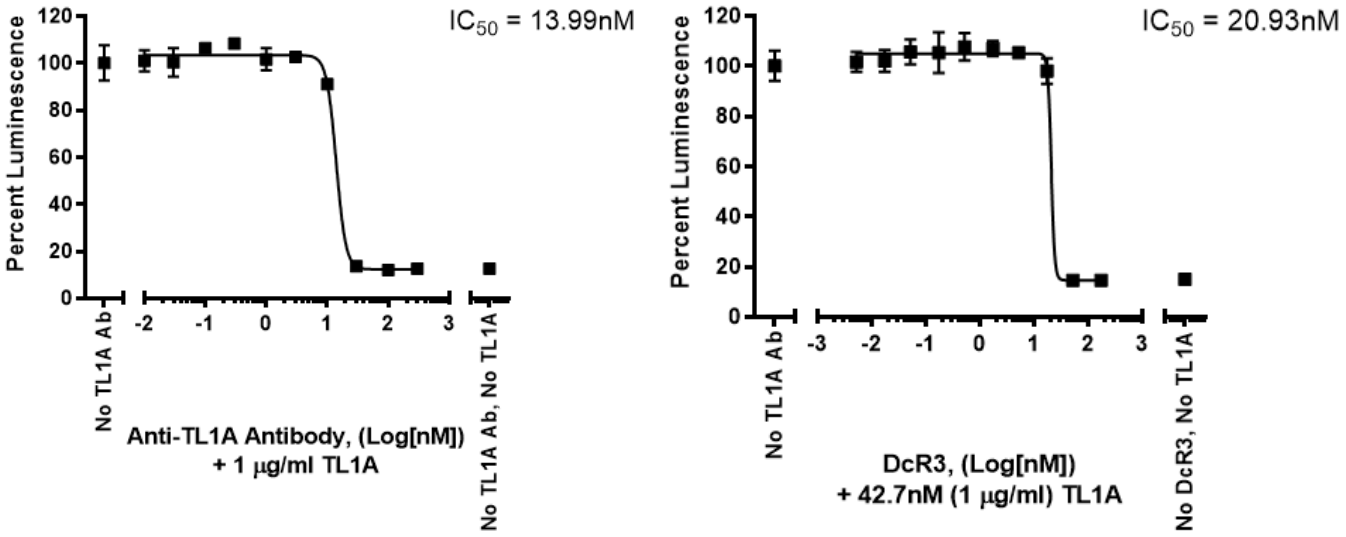

Figure 4: Inhibition of TL1A response by anti-TL1A Antibody and DcR3 Fc chimera in the TL1A Responsive Luciferase Reporter Jurkat Cell Line.
Right panel: A serial dilution of neutralizing anti-TL1A antibody (#101729) was prepared and pre-incubated with 1 µg/ml human TL1A for 1 hour. After the 1 hour incubation, the anti-TL1A antibody and TL1A mixture was added to the cells. The plate was incubated for 5 hours before being assayed with One-Step™ Luciferase Detection Reagent (#60690). Data is shown as the percent luminescence (Luminescence of TL1A-stimulated cells in the absence of antibody, defined as 100%).
Left panel: A serial dilution of DcR3 Fc chimera was prepared and pre-incubated with 1 µg/ml human TL1A for 1 hour. After the 1 hour incubation, the DcR3 Fc chimera and TL1A mixture was added to the cells. The plate was incubated for 5 hours before being assayed with One-Step™ Luciferase Detection Reagent (#60690). Data is shown as the percent luminescence (Luminescence of TL1A-stimulated cells in the absence of antibody, defined as 100%).

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

Sequence
DR3, also known as TNFRSF25; NM_003790.3

MEQRPRGCAAVAAALLLVLLGARAQGGTRSPRCDCAGDFHKKIGLFCCRGCPAGHYLKAPCTEPCGNSTCLVCPQDTFLAWEN
HHNSECARCQACDEQASQVALENCSAVADTRCGCKPGWFVECQVSQCVSSSPFYCQPCLDCGALHRHTRLLCSRRDTDCGTCL
PGFYEHGDGCVSCPTSTLGSCPERCAAVCGWRQMFWVQVLLAGLVVPLLLGATLTYTYRHCWPHKPLVTADEAGMEALTPPPA
THLSPLDSAHTLLAPPDSSEKICTVQLVGNSWTPGYPETQEALCPQVTWSWDQLPSRALGPAAAPTLSPESPAGSPAMMLQPGP
QLYDVMDAVPARRWKEFVRTLGLREAEIEAVEVEIGRFRDQQYEMLKRWRQQQPAGLGAVYAALERMGLDGCVEDLRSRLQR
GP

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

References
Xu WD, *et al.,* 2022 *Front. Immunol.* 13: 891328.

Related Products

Products	*Catalog #*	*Size*
GITR/NF-κB Luciferase Reporter Jurkat Cell Line	60651	2 vials
Firefly Luciferase Jurkat Cell Line	78373	2 vials
Human Tumor Necrosis Factor-alpha Recombinant	90244	10 µg/50 µg
Anti-TL1A Neutralizing Antibody	101729	50 µg/100 µg

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