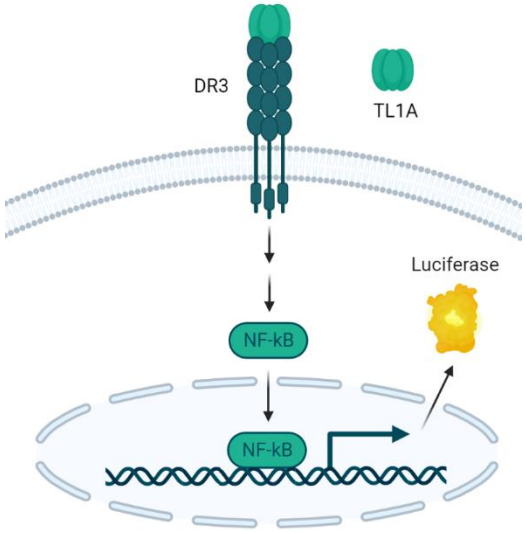
Description

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 gene is driven by NF-kB response elements located upstream of the minimal TATA promoter. Activation of the NF-kB signaling pathway by DR3 ligand TL1A (TNF-like Protein 1A; TNFSF15) can be monitored by measuring luciferase activity. This cell line has been validated for stimulation by TL1A.



Background

TNF-like ligand 1A (TL1A, also known as Vascular endothelial growth inhibitor, or VEGI) is an anti-angiogenic cytokine. It is an important mediator of inflammation, participates in innate and adaptive immune homeostasis through binding to its receptor, DR3, and 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 disorders.

Application

- Characterize the activity of soluble TL1A
- 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, Human Recombinant His-Tag	SinoBiological #17049-H07H
Growth Medium 2A	BPS Bioscience #60190
Assay Medium: Thaw Medium 2	BPS Bioscience #60184
96-well tissue culture treated, white, clear-bottom assay plate	Corning #3610
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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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 for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 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

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 **(no Geneticin or Hygromycin)**.
   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 **(no Geneticin or Hygromycin)**.

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 2 **(no Geneticin or Hygromycin)**, 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 **(contains Geneticin and Hygromycin)**.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, at no less than 0.2 x 10^6 cells/ml of Growth Medium 2A **(contains Geneticin and Hygromycin)**. 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~2 x 10^6 cells/ml.

2. Dispense 1 ml of cell aliquots into cryogenic vials. 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 storage.

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

Validation

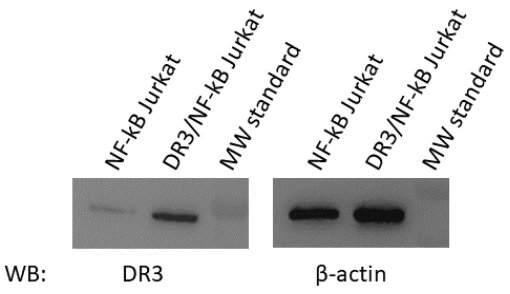


Figure 1: DR3 protein expression in parental NF-kB luciferase reporter Jurkat cells compared to DR3/NF-kB luciferase reporter Jurkat cells.
Cells were lysed and human DR3 expression levels were analyzed by SDS-PAGE electrophoresis followed by western blotting using an anti-DR3 rabbit monoclonal antibody (clone 11H6L9; Thermo Fisher #702277) and an anti-actin rabbit monoclonal antibody (clone 13E5; 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 triplicates.

A. Evaluation of soluble TL1A

1. Culture the cells so that they reach a density of ~1.5 x 10^6 cells/ml on the day before the experiment.

2. Dilute the cells with fresh growth medium 2A at 1:2 (or 1:3) ratio, so the cell density is ~0.3to 0.5 x 10^6 cells/ml (alternatively, culture the cells to a density of ~0.5 x 10^6 cells/ml on the day of the experiment and continue the next step).

3. On the day of the experiment, harvest the cells by centrifugation at 300 x g for 5 minutes.

4. After centrifugation, remove the growth medium by aspiration and resuspend the cells in pre-warmed Thaw medium 2 at a density of 0.3 x 10^6 cells/ml.

5. Dispense 90 µl/well of the resuspended cells in Thaw medium 2 in a white clear bottom 96-well plate. Keep three wells without cells for measuring the background luminescence signal (Blank).

6. Prepare an intermediate dilution of TL1A in Thaw medium 2 at a concentration 10-fold higher than the desired final concentration. Add 10 µl of the diluted TL1A to each well.

7. Add 10 µl of Thaw medium 2 in the “unstimulated” negative control wells.

8. Incubate the cells at 37°C in a CO_2 incubator for ~5 hours.

9. After ~5 hours, perform the luciferase assay using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Add 100 µl of the ONE-Step Luciferase reagent per well and rock at room temperature for ~15 minutes.

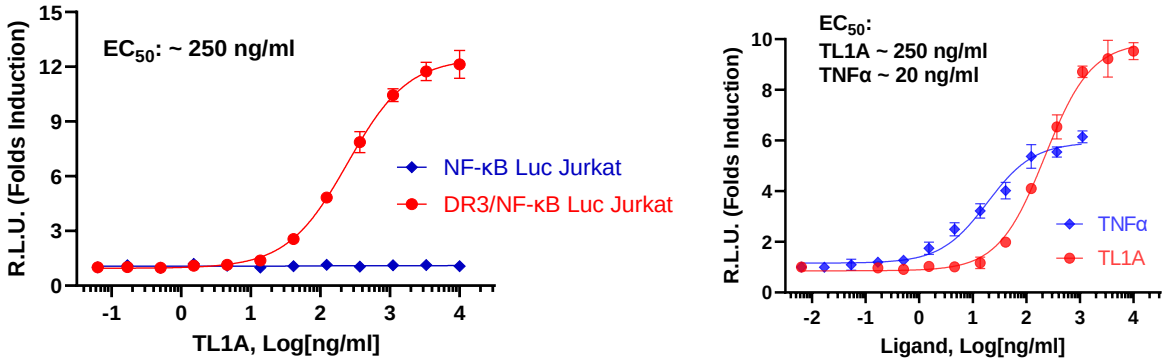


Figure 1. Dose-response of TL1A in TL1A Responsive Luciferase Reporter Jurkat cell line.
The cell-based assay was performed with increasing concentrations of TL1A or TNFα, and the luciferase reporter activity was measured using the ONE-Step™ Luciferase Assay System reagent. Signal induction was calculated based on the signal from the well containing no TL1A (set to 1). Jurkat cells that do not express DR3 (NF-κB Luciferase Reporter Jurkat cell line, BPS Bioscience #60651) do not show stimulation by TL1A (left panel).

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 all further questions, please email support@bpsbioscience.com.

References
Xu WD, *et al.* Role of TL1A in Inflammatory Autoimmune Diseases: A Comprehensive Review. *Front. Immunol.* 2022; 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	Several sizes