Description

The TL1A: DR3 Inhibitor Screening TR-FRET Assay Kit is designed for the screening and profiling of neutralizing antibodies or inhibitors of the interaction between TL1A (TNF-like ligand 1A) and DR3 (Death Receptor 3) using TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer). This kit requires no time-consuming washing steps and comes in a convenient 384-well format, with purified biotinylated-DR3 (amino acids 25-199), europium-labeled (Eu) TL1A (amino acids 72-end), Dye-labeled Acceptor, and assay buffer for 400 reactions.

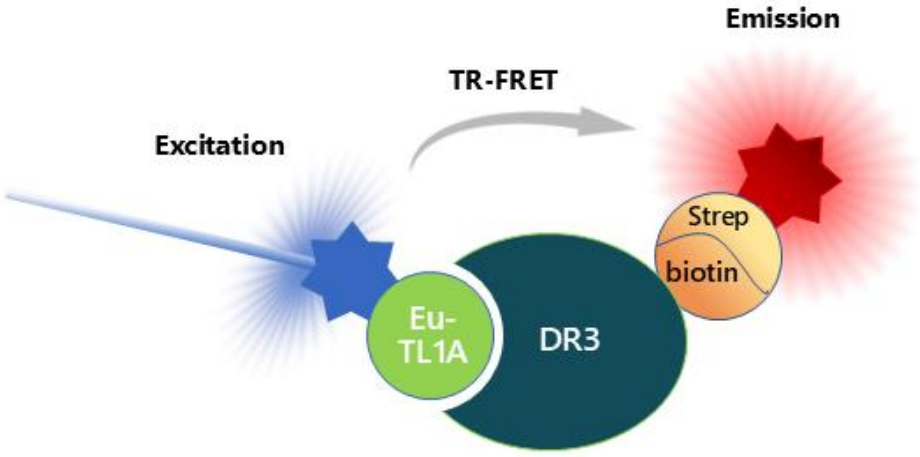

Figure 1. Illustration of the mechanism of the TL1A: DR3 Inhibitor Screening TR-FRET Assay Kit.
A sample containing europium-labeled TL1A, a dye-labeled streptavidin acceptor, biotinylated DR3, and an inhibitor, is incubated for 30 minutes. The europium-labeled TL1A binds to DR3-biotin, which receives the dye-labeled streptavidin acceptor. The TR-FRET signal is generated by proximity induced upon interaction of TL1A with DR3. Then, the fluorescence intensity is measured using a fluorescence reader capable of TR-FRET measurements. The signal generated is proportional to TL1A and DR3 binding activity.

Background

DR3 (death receptor 3), also known as tumor necrosis factor receptor superfamily member 25 or TNFRSF25, is a membrane receptor of the tumor necrosis factor receptor superfamily of proteins (TNFRSF), which associates with TL1A (TNF-like protein 1A) in T and NK cells. DR3 has been recognized as a significant anti-apoptotic and differentiation factor and it is a co-stimulatory receptor. TL1A, also called TNFSF15, is a member of the tumor necrosis factor family. It is expressed in different immune cells, such as monocyte, macrophage, dendritic cell, T cell and non-immune cells. TL1A competitively binds to DR3, having a higher affinity for DcR3 (decoy receptor 3) providing stimulatory signal for downstream signaling pathways, and then regulates proliferation, activation, apoptosis, and chemokine production in effector cells. The role of DR3 in T cell activation and consequently in cytokine secretion and cell proliferation, make it an attractive target in cancer therapy. Inhibition of DR3-TL1A interaction has substantial therapeutic potential in the treatment of solid tumors.

Application(s)

Screen or titrate small molecule inhibitors or biologics for drug discovery and high-throughput screening (HTS) applications of the TL1A binding to DR3.

Supplied Materials

Catalog #	Name	Amount	Storage
102650	TL1A, His-, Avi-Tag, Europium-Labeled*	5 µg	-80°C
102545	DR3, His-, Avi-Tag, Biotin-Labeled*	20 µg	-80°C
79716	5x PTP Buffer	3 ml	-20°C
	Dye-Labeled Acceptor	10 µl	-20°C
79969	White non-binding 384-well microplate	1	Room Temp

**The initial concentration of the proteins is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
- Adjustable micropipettor and sterile filter tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- This kit is compatible with up to 1% final DMSO concentration.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Blank”, “Positive Control” and “Test Compound” wells.
- We recommend using Anti-TL1A Neutralizing Antibody (BPS Bioscience #101729) or DcR3 (BPS Bioscience #102420) as an internal control for the assay. If not running a dose response curve for the control inhibitor, run at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1

1. Prepare **1x PTP Buffer** by diluting 5-fold the **5x PTP Buffer** with distilled water.

2. Dilute **Dye-Labeled acceptor** 200-fold in 1x PTP Buffer (5 µl/well).

   Note: Make only enough needed for the assay; store remaining stock solution in single use aliquots (minimum volume of 5 µl/ aliquot) at -20°C.

3. Thaw **TL1A-Eu** protein on ice. Briefly spin the tube to recover the full content.

4. Dilute **TL1A-Eu** protein to 2 ng/µl with 1x PTP Buffer (5 µl/well).

5. Prepare a **Master Mix** (13 µl/well): N wells × (5 µl of diluted Dye-Labeled Acceptor + 5 µl of diluted TL1A-Eu + 3 µl of 1x PTP Buffer).

6. Add 13 µl of **Master Mix** to every well.

7. Prepare the **Test Compound** (2 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 20 µl.

   7.1 If the Test Compound is water-soluble, prepare serial dilutions in 1x PTP Buffer at concentrations 10-fold higher than the desired final concentrations.

   For the positive and negative controls, use 1x PTP Buffer (Diluent Solution).

   OR

   7.2 If the Test Compound is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in 1x PTP Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using 1x PTP Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in 1x PTP Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO in the assay should not exceed 1%.

8. Add 2 µl of **Test Compound** to each well designated “Test Compound”.

9. Add 2 µl of **Diluent Solution** to the “Positive Control” and “Blank” wells.

10. Thaw **DR3-biotin** on ice. Briefly spin the tube to recover its full content.

11. Dilute **DR3-biotin** to 10 ng/µl with 1x PTP Buffer (5 µl/well).

12. Add 5 µl of **diluted DR3-biotin** to each well, except "Blank" wells.

13. Add 5 µl of **1x PTP Buffer** to the "Blank" wells.

14. Incubate at RT for 30 minutes with gentle agitation.

15. Read the TR-FRET signal in a microtiter-plate reader under settings described below (settings may need optimization depending on the instrument).

16. The "Blank" value should be subtracted from all other values.

	Blank	**Positive Control**	**Test Compound**
Master Mix	13 µl	13 µl	13 µl
1x PTP Buffer	5 µl	-	-
Test Compound	-	-	2 µl
Diluent Solution	2 µl	2 µl	-
Diluted DR3-Biotin (10 ng/µl)	-	5 µl	5 µl
Total	**20 µl**	**20 µl**	**20 µl**

Instrument Settings

Two sequential measurements should be conducted. Eu-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

Reading Mode	Time Resolved
Excitation Wavelength	317 (bandwidth 20 nm)
Emission Wavelength	620 (bandwidth 10 nm)
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	317 (bandwidth 20 nm)
Emission Wavelength	665 (bandwidth 10 nm)
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS

Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

$$FRET = \frac{S_{665}}{S_{620}}$$

When percentage activity is calculated, the FRET value from the Blank (it is expected that Blank and Negative

Control have a similar values) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\%\ Activity = \frac{FRET_S - FRET_{blank}}{FRET_P - FRET_{blank}} \times 100\%$$

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{blank}$ = FRET value for the Blank, and $FRET_p$ = FRET value for the Positive Control (no inhibitor).

Example Results

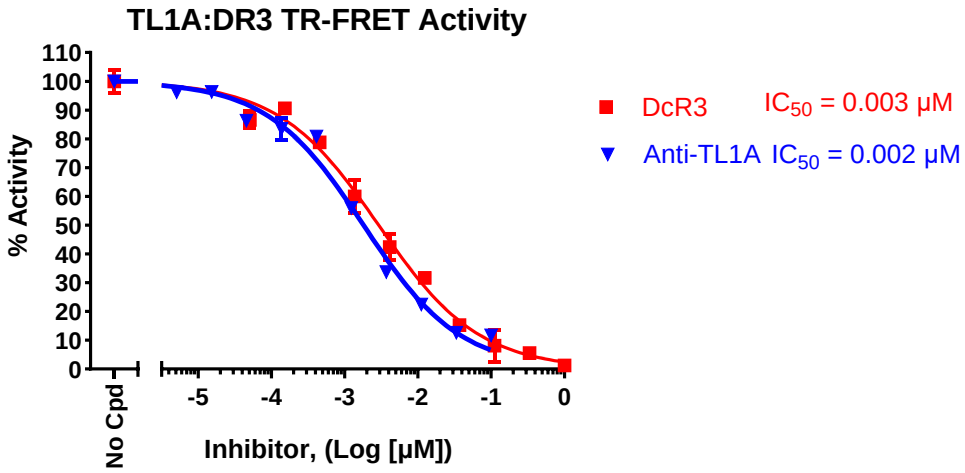

Figure 2. Inhibition of DR3:TL1A binding by Anti-TL1A Neutralizing Antibody and DcR3.
Binding between TL1A and DR3 proteins was measured in the presence of increasing concentrations of Anti-TL1A Neutralizing Antibody (#101729) or DcR3 (#102420) decoy protein. Results are expressed as percent of binding, in which the binding measured in the absence of inhibitor is set to 100%.

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
Xu, W. D., et al., 2022 Front. Immunol. 13: 1-10.
Zwolak, A., et al., 2022 Sci. Rep. 12(1): 20538.

Related Products

Products	*Catalog #*	*Size*
DR3:TL1A inhibitor Screening Assay Kit	82241	96 reactions/ 384 reactions
DcR3: TL1A Inhibitor Screening Assay Kit	82160	96 reactions
TL1A-Responsive Luciferase Reporter Jurkat Cell Line	78811	2 vials
Anti-TL1A Neutralizing Antibody	101729	50 µg/ 100 µg

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