Description

The DcR3:TL1A Inhibitor Screening Assay Kit is designed for screening and profiling inhibitors of the interaction between DcR3 (Decoy Receptor 3) and TL1A (TNF-like ligand 1A). This kit comes in a convenient 96-well format, with biotin-labeled TL1A, purified DcR3, streptavidin-labeled HRP, and assay buffer for 100 binding reactions.

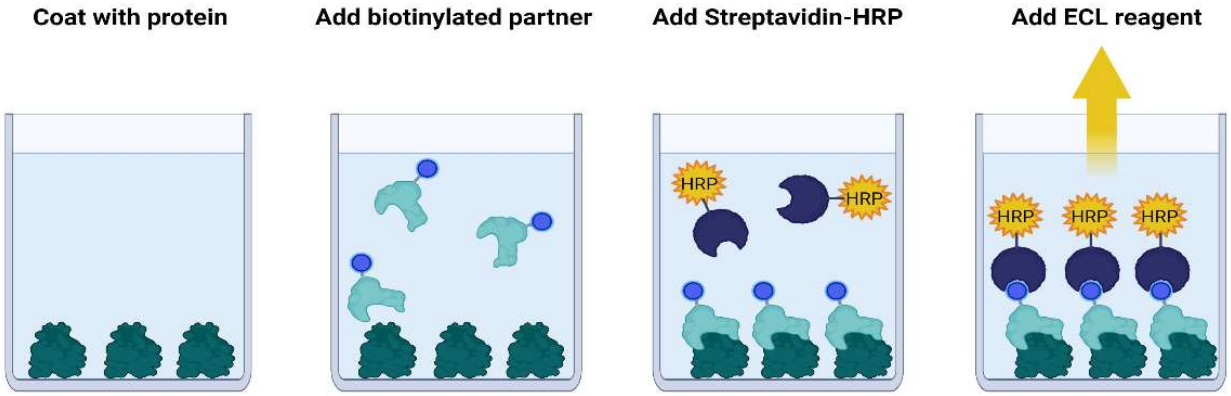

Figure 1: DcR3:TL1A Inhibitor Screening Assay Kit workflow diagram.

First, DcR3 is coated on a 96-well plate. Next, biotinylated TL1A is incubated with DcR3. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence. The chemiluminescence signal is proportional to the binding of DcR3:TL1A.

Background

Decoy receptor 3 (DcR3) is a soluble receptor of the tumor necrosis factor receptor superfamily of proteins (TNFRSF), which associates with its respective ligands, such as TL1A, FasL and LIGHT. DcR3 has been recognized as a significant anti-apoptotic factor with prominent involvement in various inflammatory and neoplastic conditions. Increased intratumor expression of DcR3 and elevated soluble DcR3 protein content in the sera of patients has been reported for various malignancies. 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 DcR3, providing stimulatory signal for downstream signaling pathways, and then regulates proliferation, activation, apoptosis, and chemokine production in effector cells. Inhibition of DcR3-TL1A interaction has substantial therapeutic potential.

Applications

Screen or titrate small molecule inhibitors or biologics for drug discovery and high-throughput screening (HTS) applications of the TL1A binding to DcR3.

Supplied Materials

Catalog #	Name	Amount	Storage
101882	TL1A, His-Tag, Avi-Tag, Biotin-Labeled*	5 µg	-80 °C
	DcR3*	25 µg	-80 °C
79311	3x Immuno Buffer 1	50 ml	-20 °C
79728	Blocking Buffer 2	50 ml	+4 °C
79742	Streptavidin-HRP	10 µl	+4 °C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	96-well white microplate	1	Room Temp

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

Materials Required but Not Supplied

- 1x PBS (Phosphate Buffer Saline)
- Luminometer or plate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Orbital shaker

Stability

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

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

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, “Uncoated Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

Step 1: Plate Coating

1. Thaw **DcR3** on ice. Briefly spin the tube to recover its full content.

2. Dilute 3-fold the **3x Immuno Buffer 1** with distilled water. This makes **1x Immuno Buffer 1.**

3. Dilute **DcR3** to 5 ng/µl in 1x Immuno Buffer 1 (50 µl/ well).
4. Add 50 µl of diluted DcR3 solution to each well and incubate overnight at 4°C.
5. Leave uncoated wells for use as the "Uncoated Control".
6. Tap the plate onto clean paper towels to remove liquid.
7. Wash the plate three times with 100 µl of 1x Immuno Buffer 1.
8. Tap the plate onto clean paper towels to remove liquid.
9. Add 100 µl of **Blocking Buffer 2** to each well.
10. Incubate for 1 hour at Room Temperature (RT).
11. Tap the strip onto clean paper towels to remove liquid.

Step 2: Reaction

1. Prepare a Master Mix (25 µl/well): N wells x (10 µl of 3x Immuno Buffer 1 + 15 µl of distilled water).
2. Add 25 µl of Master Mix to each well.
3. Prepare the Test inhibitor (5 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 50 µl.

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

   OR

   3.2 If the Test inhibitor 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 Immuno Buffer 1 to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using 1x Immuno Buffer 1 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 Immuno Buffer 1 (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%.

4. Add 5 µl of Test inhibitor to each well designated "Test Inhibitor".

5. Add 5 µl of Diluent Solution to the "Positive Control", "Uncoated Control", and "Blank" wells.
6. Incubate at RT for 30 minutes with gentle agitation.
7. Thaw **TL1A**-biotin on ice. Briefly spin the tube to recover its full content.
8. Dilute TL1A-biotin **to** 0.15 ng/µl with 1x Immuno Buffer 1 (20 µl/well).
9. Add 20 µl of diluted enzyme to the "Positive Control", "Ligand Inhibitor and "Test Inhibitor" wells.
10. Add 20 µl of 1x Immuno Buffer 1 to the "Blank" wells.
11. Incubate at RT for 1 hour with gentle agitation.
12. Tap the plate onto clean paper towels to remove liquid.
13. Wash the plate three times with 100 µl of 1x Immuno Buffer 1.
14. Tap the plate onto clean paper towels to remove liquid.
15. Add 100 µl of **Blocking Buffer 2** to each well.
16. Incubate for 10 minutes at RT.
17. Tap the strip onto clean paper towels to remove liquid.

Step 3: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 2.
2. Add 100 µl of diluted Streptavidin-HRP to each well.
3. Incubate for 1 hour at RT with gentle agitation.
4. Wash three times with 200 µl of 1x Immuno Buffer 1.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Add 100 µl of Blocking Buffer 2 to every well.
7. Incubate for 10 minutes at RT with gentle agitation.
8. Tap the plate onto clean paper towel to remove the liquid.
9. Just before use, mix 50 µl of **ELISA ECL Substrate A** with 50 µl of **ELISA ECL Substrate B** (100 µl/well of mix is needed).

10. Add 100 µl to each well.

11. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

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

Component	Blank	Uncoated Control	Positive Control	Test Inhibitor
Master Mix	25 µl	25 µl	25 µl	25 µl
Test Inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
	30 minutes at Room Temperature			
Diluted TL1A-Biotin (0.15 ng/ µl)	-	20 µl	20 µl	20 µl
1x Immuno Buffer 1	20 µl	-	-	-
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Validation Data

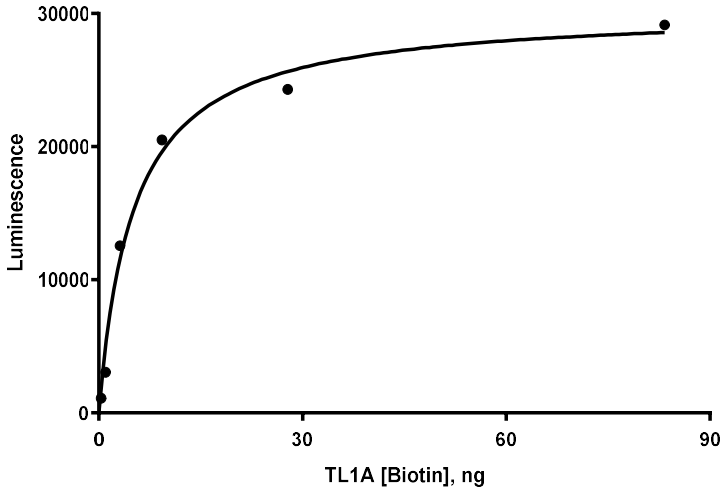

Figure 2. DcR3-TL1A binding activity.
Binding of DcR3 and TL1A was measured in the presence of increasing concentrations of TL1A-biotin. Luminescence was measured using a Bio-Tek fluorescent microplate reader.

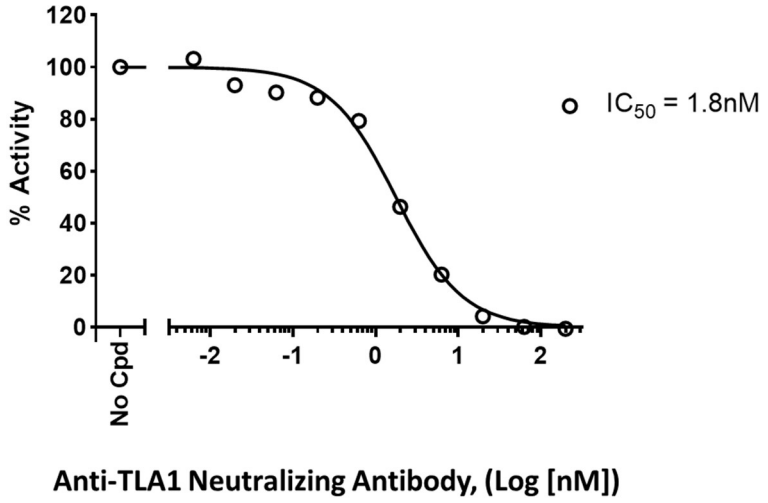

Figure 3. Inhibition of DcR*3-TL1A binding activity by Anti-TL1A Neutralizing Antibody.*
Binding activity was measured in the presence of increasing concentrations of Anti-TL1A Neutralizing Antibody (BPS Bioscience #101729). Results are expressed as percent binding, in which the binding measured in the absence of inhibitor is set to 100%.

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

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Lagou,S., *et al*., 2022 *Cancer Diagn. Progn.* 2(4): 411-421.
Xu, W.-D., *et al., 2022 Front. Immunol.* 13: 1-10.

Related Products

Products	*Catalog #*	*Size*
TL1A-Responsive Luciferase Reporter Jurkat Cell Line	78811	2 vials
Anti-TL1A Neutralizing Antibody	101729	50 µg/100 µg
TL1A, His-Tag- Avi-Tag Recombinant	101880	100 µg/400 µg
LIGHT, His-Tag (Human) Recombinant	71266	100 µg
LIGHT: HVEM [Biotinylated] Inhibitor Screening Assay Kit	79684	96 reactions

Version 11/9/2023