SZABO SCANDIC

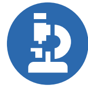
Part of Europa Biosite

Produktinformation

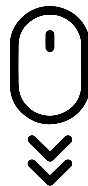
Forschungsprodukte & Biochemikalien

Zellkultur & Verbrauchsmaterial

Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The DR3:TL1A Inhibitor Screening Assay Kit is designed for screening and profiling inhibitors of the interaction between DR3 (Death Receptor 3) and TL1A (TNF-like ligand 1A). This kit comes in a convenient 96-well format, with biotin-labeled TL1A, purified DR3, streptavidin-labeled HRP, and assay buffer for 100 binding reactions.

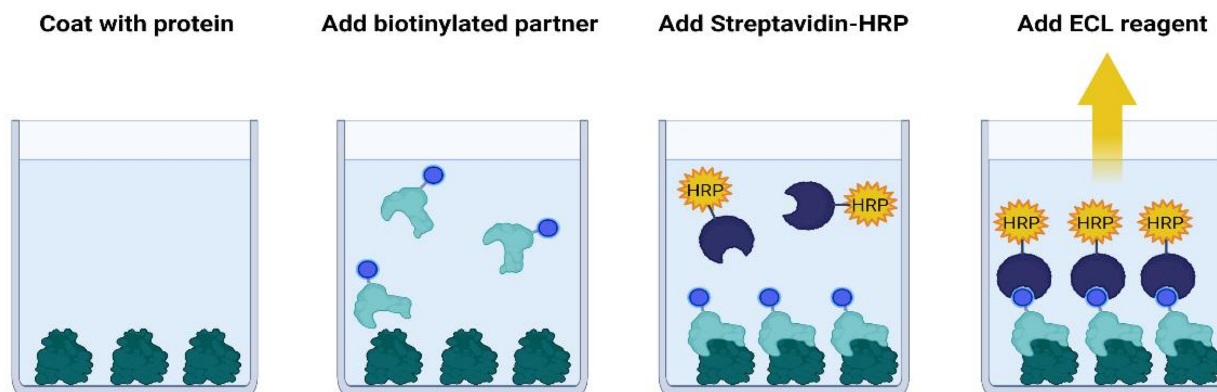


Figure 1: DR3:TL1A Inhibitor Screening Assay Kit workflow diagram.
First, DR3 is coated on a 96-well plate. Next, biotinylated TL1A is incubated with DR3. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence. The chemiluminescence signal is proportional to the binding of DR3:TL1A.

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. It 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, makes it an attractive target in cancer therapy. Inhibition of DR3-TL1A interaction has substantial therapeutic potential in the treatment of solid tumors.

Applications

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

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Supplied Materials

Catalog #	Name	Amount	Storage
101882	TL1A, His-Tag, Avi-Tag, Biotin-Labeled*	5 µg	-80 °C
	DR3*	25 µg	-80 °C
	5x PP-02 Buffer	4 ml	-20 °C
	Blocking Buffer 7	40 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)
- PBST Buffer (1x PBS containing 0.05% Tween-20)
- 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" (uncoated wells, for background determination), "Positive 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).
- We recommend using Anti-TL1A Neutralizing Antibody (BPS Bioscience #101729) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

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Step 1: Plate Coating

1. Thaw **DR3** on ice. Briefly spin the tube to recover its full content.
2. Dilute **DR3** to 5 ng/µl in 1x PBS (50 µl/well).
3. Add 50 µl of diluted DR3 solution to each well, except "Blank" wells.
4. Add 50 µl of PBS to the "Blank" wells.
5. Incubate overnight at 4°C.
6. Wash the plate three times with 150 µl of PBST.
7. Tap the plate onto clean paper towels to remove liquid.
8. Add 100 µl of **Blocking Buffer 7** to each well.
9. Incubate for 90 minutes at Room Temperature (RT).
10. Wash the plate three times with 150 µl of PBST.
11. Tap the plate onto clean paper towels to remove liquid.

Step 2: Reaction

1. Prepare **1x PP-02 Buffer** by diluting 5-fold the **5x PP-02 Buffer** with distilled water.
2. Add 20 µl of 1x PP-02 Buffer 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 PP-02 Buffer 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 PP-02 Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using 1x PP-02 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.

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For positive and negative controls, prepare 10% DMSO in 1x PP-02 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%.

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

5. Add 5 µl of Diluent Solution to the "Positive 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 2 ng/µl with 1x PP-02 Buffer (25 µl/well).

9. Add 25 µl of diluted enzyme to each well.

10. Incubate at RT for 1 hour with gentle agitation.

11. Wash the plate three times with 150 µl of PBST.

12. Tap the plate onto clean paper towels to remove liquid.

13. Add 100 µl of **Blocking Buffer 7** to each well.

14. Incubate for 10 minutes at RT.

15. Tap the plate onto clean paper towels to remove liquid.

Step 3: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 7 (50 µl/well).

2. Add 50 µl of diluted Streptavidin-HRP to each well.

3. Incubate for 1 hour at RT with gentle agitation.

4. Wash the plate three times with 150 µl of PBST.

5. Tap the plate onto clean paper towel to remove the liquid.

6. 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).

7. Add 100 µl to each well.

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

9. The “Blank” value should be subtracted from all other values.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
1x PP-02 Buffer	20 µl	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
	30 minutes at Room Temperature		
Diluted TL1A-Biotin (2 ng/ µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

Validation Data

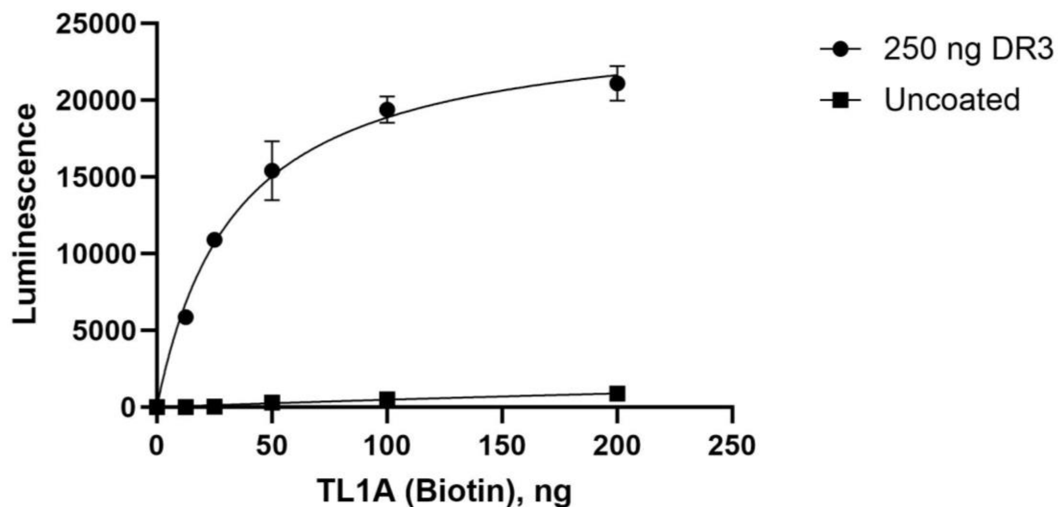

Figure 2. DR3:TL1A binding.
Binding of DR3 and TL1A was measured in the presence of increasing concentrations of TL1A-biotin. TL1A was incubated with either 250 ng/well of DR3 or no DR3 (uncoated condition). Chemiluminescence was measured using a Bio-Tek fluorescent microplate reader.

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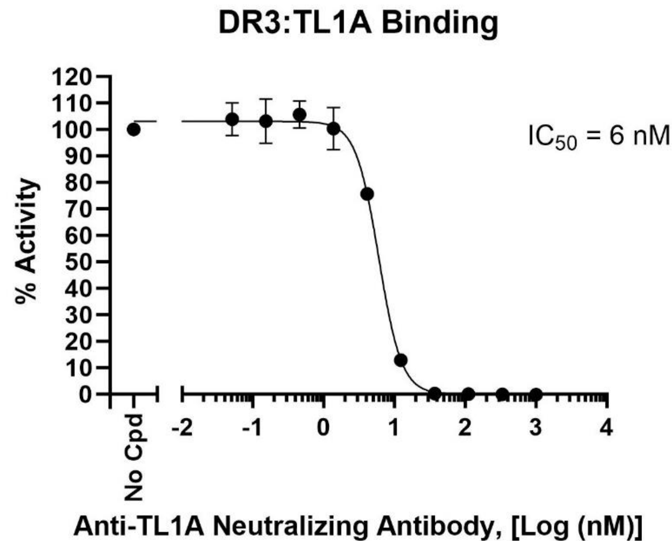

Figure 3. Inhibition of DR3:TL1A binding by Anti-TL1A Neutralizing Antibody.
Binding was measured in the presence of increasing concentrations of Anti-TL1A Neutralizing Antibody (BPS Bioscience #101729). Results are expressed as percent of binding, in which the binding measured in the absence of inhibitor is set to 100%.

Data shown is representative. 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
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*
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
TL1A, His-Tag- Avi-Tag Recombinant	101880	100 µg/500 µg
TL1A, His-Tag- Avi-Tag, Biotin-Labeled Recombinant	101882	100 µg/500 µg

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