Description

The CD155:TIGIT [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is a ELISA-based assay designed for screening and profiling molecules that block the binding between CD155 (cluster of differentiation 55) and TIGIT (T-cell immunoreceptor with Ig and ITIM domains). This kit comes in a convenient 96-well format, with enough recombinant human biotin-labeled TIGIT (amino acids 22-141), CD155 (amino acids 27-343), streptavidin-labeled HRP, and assay buffer for 100 reactions.

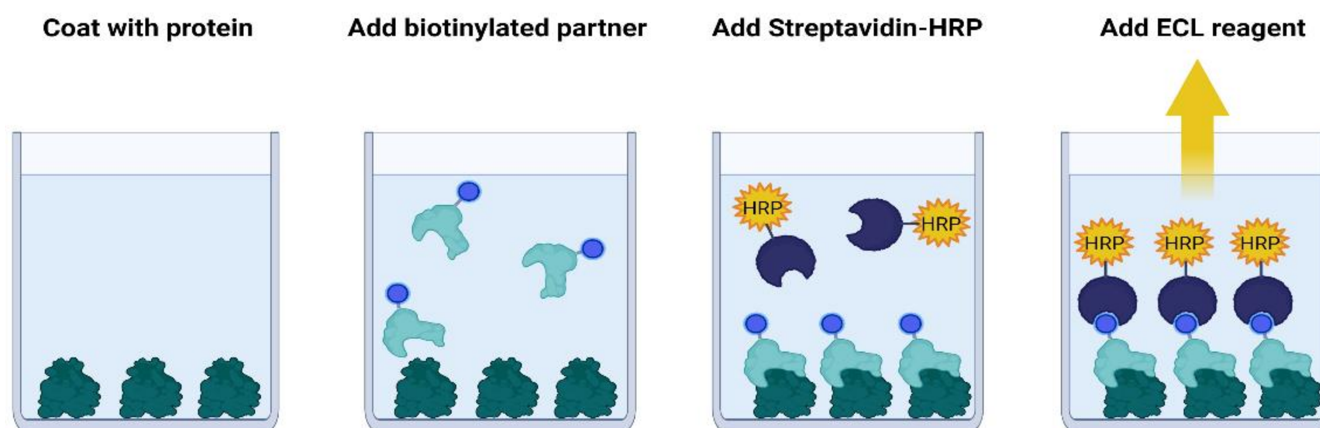

Figure 1: Illustration of the mechanism of CD155: TIGIT [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit.

A 96-well plate is coated with the human recombinant CD155 protein. After blocking, the plate is pre-incubated with an inhibitor or neutralizing antibody. After incubation with Biotin-TIGIT, the plate is washed and Streptavidin-HRP is added. The ELISA ECL substrate is added, and the resulting signal is measured using a chemiluminescence microplate reader. The chemiluminescence signal is proportional to the binding of CD155 to TIGIT.

Background

TIGIT (T-cell immunoreceptor with Ig and ITIM domains) is an immune checkpoint, co-inhibitory receptor of the family of PVR (poliovirus receptor)-like proteins that is highly expressed on the surface of Natural Killer (NK) cells, activated $CD4^+$, $CD8^+$, and regulatory T cells. TIGIT has three ligands: CD155, CD112 and CD113. TIGIT has the highest affinity for CD155, which is present on antigen presenting cells (APCs) such as dendritic cells, on T cells, B cells, and macrophages. TIGIT binding to CD155 recruits the Src homology (SH) domain-containing protein tyrosine phosphatases SHP1 and SHP2 or the inositol phosphatases SHIP1 and SHIP2 to the TIGIT ITIM domain (immunoreceptor tyrosine-based inhibitory motif). This increases the production of IL-10 and decreases IL-12 release, and it suppresses NF-kB (nuclear factor kappa-light chain enhancer of activated B cells) and NFAT (nuclear factor of activated T cells) T cell receptor (TCR) signaling, which blocks T cell proliferation and cytokine production. TIGIT may also play a role in immune regulation via cell-intrinsic mechanisms, with anti-TIGIT agonist antibodies blocking the anti-CD3/anti-CD28 activation of T cells and DNAM-1 (DNAX accessory molecule-1) signaling in the absence of APCs. TIGIT is found at high levels in TILs (tumor-infiltrating lymphocytes) in AML (acute myeloid leukemia), MM (multiple myeloma), NSCLC (non-small cell lung carcinoma) and other cancers. On the other hand, cancer cells can also express CD155. The development of TIGIT-blocking strategies is an active area of research, with several ongoing clinical trials using anti-TIGIT blocking antibodies.

Application(s)

Screen or titrate small molecule inhibitors or antibodies that block CD155 binding to TIGIT for drug discovery and high-throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
71251	TIGIT, Fc Fusion, Avi-Tag, Biotin-Labeled*	5 µg	-80°C
79063	CD155, Fc-Avi-Tag*	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 Substrates A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrates B (brown bottle)	6 ml	Room Temp
79699	White 96-well 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 Buffer (Phosphate Buffer Saline, pH 7.4)
- PBS-T Buffer (1x PBS buffer with 0.05% Tween-20)
- Microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile 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

The DMSO concentration in the final reaction should be ≤1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Non-Coated Control", "Blank", "Positive Control" and "Test Compound" wells.
- We recommend pre-incubating antibodies or protein inhibitors with the target protein prior to the addition of the binding partner.
- For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.
- We recommend using Anti-TIGIT Inhibitor Antibody (#71218) 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.

- We recommend maintaining the diluted proteins 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 - Plate coating with CD155

Coat the plate one day prior to running your samples.

1. Thaw **CD155** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **CD155** protein to 5 ng/µl in PBS (50 µl/well).
3. Add 50 µl of diluted **CD155** protein solution to each well, except “Non-Coated Control” wells.
4. Add 50 µl of PBS to the “Non-Coated Control” wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times with 150 µl of PBS-T Buffer per well.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Add 100 µl of Blocking Buffer 7 to every well.
9. Incubate for 90 minutes at Room Temperature (RT) with gentle agitation.
10. Tap the plate onto clean paper towel to remove the liquid.
11. Start your testing immediately.

Step 2: Assessment of inhibition/blocking of CD155 binding to TIGIT by blocking molecules.

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 “Non-Coated Control”, “Positive Control” and “Test Compound” wells.
3. Add 45 µl of 1x PP-02 Buffer to “Blank” wells.
4. Prepare the Test Compound (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.

   4.1 If the Test Compound is water-soluble, prepare serial dilutions in 1x PP-02 Buffer at concentrations 10- fold higher than the desired final concentrations.

   OR

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4.2 If the Test Compound is soluble in DMSO, prepare the compound 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.

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%.

5. Add 5 µl of the test compound to each well designated “Test Compound”.

6. Add 5 µl of Diluent Solution to the “Blank”, “Non-Coated Control” and “Positive Control” wells.

7. Incubate the plate for 15-30 minutes at RT with gentle agitation.

8. Thaw the **TIGIT-Biotin** on ice. Briefly spin the tube to recover the full content.

9. Dilute **TIGIT-Biotin** to 2 ng/µl with 1x PP-02 Buffer (25 µl/well).

10. Add 25 µl of diluted **TIGIT-Biotin** to the “Non-Coated Control, “Positive Control,” and “Test Compound” wells.

11. Incubate the plate at RT for 1 hour with gentle agitation.

	Blank	**Non-Coated Control**	**Positive Control**	**Test Compound**
PP-02 Buffer	45 µl	20 µl	20 µl	20 µl
Test Compound	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
Pre-incubate 15-30 minutes at RT				
Diluted TIGIT-Biotin (2 ng/µl)	-	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

12. Wash the plate three times with 150 µl of PBS-T Buffer per well.

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

14. Block by adding 100 µl of Blocking Buffer 7 to every well and incubate for 10 minutes at RT.

15. Tap the plate onto clean paper towel to remove the 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 the plate for 1 hour at RT with gentle agitation.

4. Wash the plate three times with 150 µl of PBS-T Buffer per well.

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

6. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).

7. Add 100 µl of mix to each well.

   Note: Discard any unused chemiluminescent mix after use.

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

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

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

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Validation Data

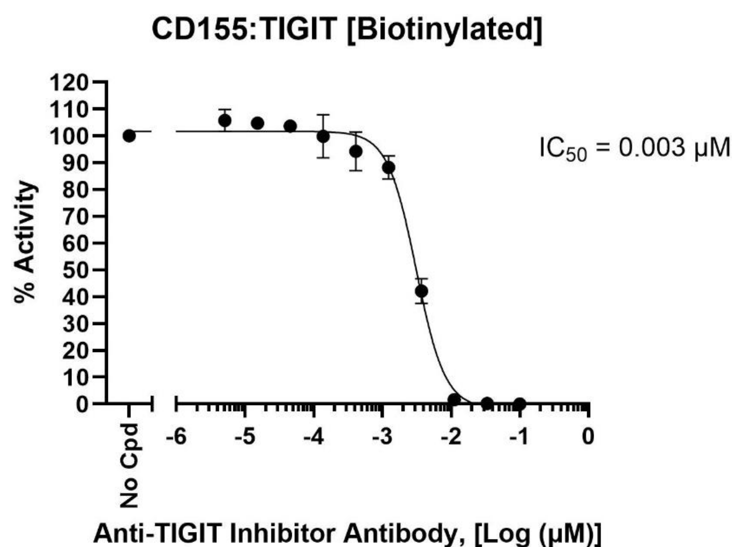

Figure 2. Inhibition of CD155 binding to TIGIT by Anti-TIGIT Inhibitor Antibody.
CD155:TIGIT binding was measured in the presence of increasing concentrations of Anti-TIGIT Inhibitor Antibody (#71218). Results are expressed as percent of binding activity, in which the binding measured in the absence 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

Yu X., *et al.*, 2009. *Nat. Immunol.* 10(1): 48-57.
Stanietsky N., *et al.*, 2009. *Proc. Natl. Acad. Sci.* 106(42): 17858-17863.
Harjunpaa H. and Guillerey C., 2020 *Clin Exp Immunol* 200(20:108-119.

Related Products

Products	*Catalog #*	*Size*
TIGIT HEK293 Cell Line	79332	2 vials
TIGIT / NFAT Reporter – Jurkat Cell Line	60538	2 vials
CD155 / TCR Activator – CHO Cell Line	60548	2 vials
CD155 (PVR)- HEK293 Cell Line	60537	2 vials
TIGIT:CD155 Homogeneous Assay Kit	72029	384 reactions
TIGIT:CD112 Homogeneous Assay Kit	72030	384 reactions

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