TNFR2: TNF-alpha[Biotinylated] Inhibitor Screening Assay Kit

#79756
96 reactions

Description

The TNFR2: TNFalpha[Biotinylated] Inhibitor Screening Assay Kit is designed to measure the binding of TNFR2 (tumor necrosis factor receptor 2) to TNF-alpha (TNFα) for screening and profiling applications. The TNFR2:TNF-alpha [Biotinylated] Inhibitor Screening Assay Kit comes in a convenient 96-well format, with enough recombinant purified biotinylated TNF-alpha (amino acids 29-216), TNFR2 (amino acids 23-257), blocking and assay buffer and detection reagents for 100 enzyme reactions.

Background

Tumor Necrosis Factor Receptor 2 (TNFR2, also known as TNFRSF1B or CD120b) is a transmembrane receptor of the TNF protein superfamily that binds the pleiotropic pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-alpha). TNFR2 can be found in several T cell subsets such as regulatory $CD8^+$ T cells (Tregs) and $CD4^+$ tumor-infiltrating T cells, myeloid lineage cells and some cancer types, and it is involved in autoimmune diseases, graft versus host disease and cancer. It has become an attractive target for cancer immunotherapy, where its different functions as oncogene and immune regulator are being explored. TNFR2 also exhibits neuroprotective properties and promotes tissue regeneration, making it a promising potential therapeutic target for the treatment of Alzheimer's disease.

Applications

Screening of small molecules and antibodies that inhibit binding of TNFR2 to TNF-alpha.

Supplied Materials

Catalog #	Name	Amount	Storage
79363	TNFR2, Fc-Fusion (IgG1), His-Avi-Tag*	10 µg	-80°C
	TNF-alpha, Biotin-Labeled*	2 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 the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- 1x PBS (phosphate buffer saline) Buffer
- Luminometer or 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

This kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Positive Control", "Ligand 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-TNF-alpha antibody 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.

Step 1: Coat plate

1. Thaw **TNFR2** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.
2. Dilute TNFR2 to 2 µg/ml with PBS (50 µl/well, except "Ligand Control" wells).
3. Add 50 µl of diluted TNFR2 to each well, except "Ligand Control" wells.
4. Incubate at 4°C overnight.
5. Dilute 3-fold the 3x Immuno Buffer 1 with distilled water. This makes 1x Immuno Buffer 1.

   Note: 30 ml of 1x Immuno Buffer 1 are enough for a 96-well plate. 3x Immuno Buffer 1 is necessary for other steps in the protocol.
6. Tap the plate onto clean paper towel to remove the liquid.
7. Wash the plate three times using 100 µl of 1x Immuno Buffer 1 per well.
8. Tap the plate onto clean paper towel to remove the liquid.
9. Block each well with 100 µl of **Blocking Buffer 2**.
10. Incubate for 1 hour at Room Temperature (RT).

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

12. Wash plate three times with 100 µl/well of 1x Immuno Buffer 1.

13. Tap the plate onto clean paper towel to remove the 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 every 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 soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration in 1x Immuno Buffer 1.

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

   OR

   3.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with 1x Immuno Buffer 1 (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

   Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Immuno Buffer 1 to keep the concentration of DMSO constant.

   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 should not exceed 1%.

4. Add 5 µl of Test Inhibitor to each well labeled as “Test Inhibitor”.

5. Add 5 µl of Diluent Solution to the “Positive Control”, “Ligand Control” and “Blank” wells.

6. Incubate at RT for 1 hour with slow agitation.

7. Thaw **TNF-alpha[biotin]** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.

8. Dilute TNF-alpha to **0.5 ng/µl** with 1x Immuno Buffer 1 (20 µl/well).

9. Initiate the reaction by adding 20 µl of diluted TNF-alpha to the wells designated “Positive Control”, “Ligand Control” and "Test Inhibitor."

10. Add 20 µl of 1x Immuno Buffer 1 to the “Blank” wells.

11. Incubate at RT for 1 hour with slow agitation.

	Blank	Ligand Control (uncoated)	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	-
1x Immuno Buffer 1	20 µl	-	-	-
Diluted TNF-alpha (0.5 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

12. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 and tap the plate onto clean paper towel.

13. Block the wells by adding 100 µl of Blocking Buffer 2 to every well.

14. Incubate for 10 minutes at RT with slow agitation.

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

16. Wash plate three times with 100 µl/well of 1x Immuno Buffer 1.

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

Step 3: Detection

1. Dilute Streptavidin-HRP 1000-fold in Blocking Buffer 2 (100 µl/well).

2. Add 100 µl of diluted Streptavidin-HRP to each well.

3. Incubate for 1 hour at RT.

4. Wash three times with 100 µl of 1x Immuno Buffer 1 and tap the plate onto clean paper towel.

5. Block the wells by adding 100 µl of Blocking Buffer 2 to every well.

6. Incubate at RT for 10 minutes.

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

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

9. Add 100 µl of mix per well.

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

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

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).

Example Results

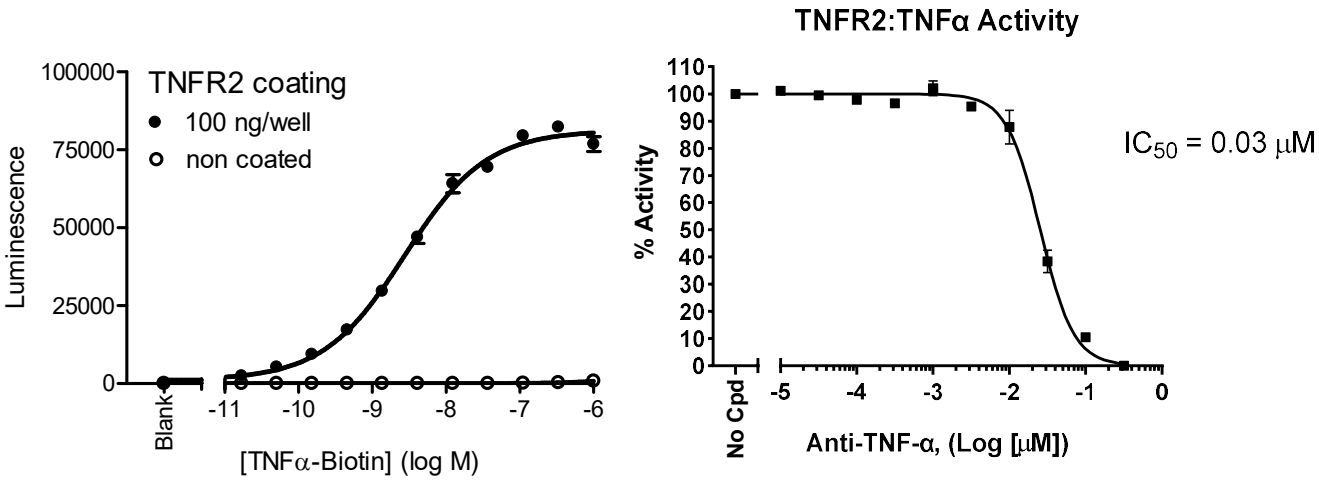

Figure 1: Binding of TNF-alpha (TNFα) to TNFR2 and inhibition of binding by an anti-TNF-alpha antibody.

A plate was coated with TNFR2, followed by incubation with increasing concentrations of TNF-alpha (top panel). Similarly, a plate was coated with TNFR2, and then incubated with a fixed amount of TNF-alpha in the presence of increasing concentrations of Human TNF-alpha Antibody (R&D Systems #AF0210-NA) (bottom panel). Luminescence was measured using a Bio-Tek microplate reader.

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

References

Vanamee ES., *et al.*, 2017 *Trends. Mol. Med.* 23(11): 1037-1046
Chen X., *et al.*, 2017 *Sci. Signal.* 10(462): eaal2328
Ortí-Casañ. N., *et al.*, 2019 *Front. Neurosci.* 13: 49.
Medler J, *et al.*, 2022 *Cancers* 14(11): 2603

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
TNFR2 Hek293 Cell Line	78828	2 vials
TNFR2 Lentivirus	78765	500 µl x 2
TNFR2, Fc-Fusion (IgG1), His-Avi-Tag, Biotin-Labeled Recombinant	100205	25 µg/50 µg
Human Tumor Necrosis Factor-beta Recombinant	90245	5 µg/10 µg/20 µg
Human Tumor Necrosis Factor-alpha Recombinant	90244	10 µg

Version 020124