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Data sheet

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

Catalog #79756
Size: 96 reactions

BACKGROUND: Tumor necrosis factor receptor 2 (TNFR2, TNFRSF1B or CD120b) is a membrane receptor that binds the pleiotropic pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-alpha). TNFR2 is predominately expressed in cells of the immune system, especially regulatory T cells, and by endothelial cells. TNFR2 exhibits neuroprotective properties and promotes tissue regeneration, making it a promising potential therapeutic target for the treatment of Alzheimer's disease, autoimmune diseases and cancer.

DESCRIPTION: The *TNFR2:TNF-alpha[Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of TNFR2:TNF-alpha. This kit comes in a convenient 96-well format, with biotin-labeled TNF-alpha, purified TNFR2, streptavidin-labeled HRP, and assay buffer for 96 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled TNF-alpha by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, TNFR2 is coated on a 96-well plate. Next, biotinylated TNF-alpha is incubated with TNFR2 on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which can be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
79363	TNFR2, Fc-Fusion (IgG1), His-Avi-Tag	10 µg	-80 °C	Avoid multiple freeze/thaw cycles!
	TNF-alpha, Biotin	2 µg	-80 °C	
79311	3x Immuno Buffer 1	50 ml	-20 °C	
79728	Blocking Buffer 2	50 ml	+4 °C	
79742	Streptavidin-HRP	15 µl	-20 °C	
79670	ELISA ECL Substrate A	6 ml	RT	
	ELISA ECL Substrate B	6 ml	RT	
79699	96-well white microplate	1	RT	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips

APPLICATIONS: This kit is useful for screening for inhibitors of TNF-alpha binding to TNFR2.

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STABILITY: Up to 6 months from date of receipt, when stored as recommended.

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

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Coating the plate with TNFR2:

1) Thaw **TNFR2** on ice. Upon first thaw, briefly spin tube containing **TNFR2** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **TNFR2** in aliquots at -80 °C. Note: **TNFR2** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **TNFR2** to 2 ng/µl in PBS.
3) Add 50 µl of diluted **TNFR2** solution to each well and incubate overnight at 4°C. Leave a couple of wells empty (uncoated), for use with the "Ligand Control" (see below).
4) Dilute **3x Immuno Buffer 1** to **1x Immuno Buffer 1** with water. (Keep a portion of the **3x Immuno Buffer 1** undiluted, for use in Step 1 below).
5) Decant to remove supernatant. Wash the plate three times with 100 µl **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.
6) Block wells by adding 100 µl of **Blocking Buffer** to each well. Incubate for 1 hour at room temperature with slow shaking. Remove supernatant as described in step 5.

Step 1:

1) Prepare the master mixture: N wells × (10 µl **3x Immuno Buffer 1** + 15 µl distilled water).
2) Add 25 µl of master mixture to each well. Use uncoated wells for the "Ligand Control."
3) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor." For the "Positive Control," "Ligand Control," and "Blank," add 5 µl of **1x Immuno Buffer 1** in 10% DMSO (inhibitor buffer). Incubate at room temperature for one hour with slow shaking. *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.*
4) Thaw **TNF-alpha-biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **TNF-alpha-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at - 80 °C. Note: ***TNF-alpha-***

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biotin *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

5) Dilute **TNF-alpha-biotin** to 0.5 ng/µl (25 nM) in **1x Immuno Buffer 1**. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

6) Add 20 µl of **1x Immuno Buffer 1** to the well designated "Blank."

	Blank	Ligand Control	Positive Control	Test Inhibitor
3x Immuno Buffer 1	10 µl	10 µl	10 µl	10 µl
Distilled water	15 µl	15 µl	15 µl	15 µl
Test Inhibitor	-	-	-	5 µl
1x Immuno Buffer 1 in 10% DMSO (Inhibitor buffer)	5 µl	5 µl	5 µl	-
1x Immuno Buffer 1	20 µl	-	-	-
TNF-alpha-biotin (0.5 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

7) Initiate reaction by adding 20 µl of diluted **TNF-alpha-biotin** (see Step 1-5) to wells labeled "Positive Control," "Ligand Control," and "Test Inhibitor". Incubate at room temperature for one hour with slow shaking.

8) Decant to remove supernatant. Wash the plate 3 times with 100 µl/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

9) Block wells by adding 100 µl of **Blocking Buffer** to each well. Incubate for 10 minutes at room temperature. Remove supernatant as in Step 1-8.

Step 2:

1) Dilute **Streptavidin-HRP** 1000-fold with **Blocking Buffer**.

2) Add 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.

3) Wash plate three times with **1x Immuno Buffer 1**. Tap plate onto clean paper towel to remove liquid.

4) Block wells by adding 100 µl of **Blocking Buffer** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.

5) Just before use, mix on ice 50 µl **ELISA ECL Substrate A** and 50 µl **ELISA ECL Substrate B**, then add 100 µl to each well. Discard any unused chemiluminescent reagent after use.

6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

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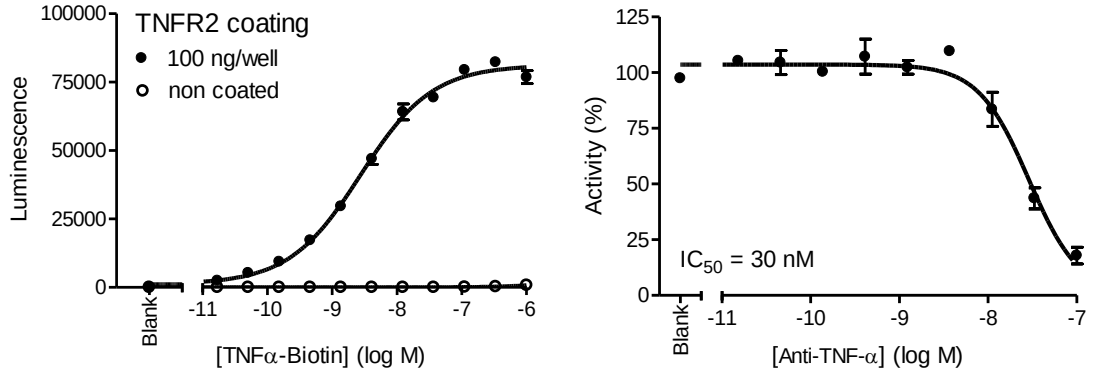
7) **Reading Chemiluminescence:**

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 milliseconds. 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 of assay results:



TNFR2:TNF-alpha binding activity, measured using the using the *TNFR2:TNF-alpha[Biotinylated] Inhibitor Screening Assay Kit*, BPS Bioscience #79756 (left). Inhibition of TNFR2:TNF-alpha binding using human TNF-alpha antibody (R&D Systems #AF-210-SP) in the *TNFR2:TNF-alpha[Biotinylated] Inhibitor Screening Assay Kit* (right). Luminescence was measured using a Bio-Tek fluorescent microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

RELATED PRODUCTS:

Product Name	**Catalog#**	**Size**
TNFR2, Fc-Fusion (IgG1), His-Avi-Tag	79363	100 µg
TNFR2, Fc-Fusion (IgG1), His-Avi-Tag, Biotin-labeled	100205	50 µg
Human Tumor Necrosis Factor-alpha	90244-A	10 µg
Human Tumor Necrosis Factor-alpha	90244-B	50 µg
Mouse Tumor Necrosis Factor-alpha	90246-A	5 µg
Mouse Tumor Necrosis Factor-alpha	90246-B	20 µg
Human Tumor Necrosis Factor-beta	90245-A	5 µg
Human Tumor Necrosis Factor-beta	90245-B	20 µg

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TROUBLESHOOTING GUIDE

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	TNFR2 or TNF-alpha-biotin has lost activity	Proteins lose activity upon repeated freeze/thaw cycles. Use fresh TNF-alpha-biotin and fresh TNFR2 (BPS Bioscience #79363). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in PBST
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of TNF-alpha-Biotin to create a standard curve