Description

The OX40[Biotinylated]:OX40L Inhibitor Screening Assay Kit is designed to measure the binding of OX40 to OX40L (OX40 ligand) for screening and profiling applications. The OX40[Biotinylated]:OX40L Inhibitor Screening Assay Kit comes in a convenient 96-well format, with enough recombinant purified biotinylated OX40 (amino acids 29-216), OX40L (amino acids 51-183), blocking and assay buffer and detection reagents for 100 enzyme reactions.

Background

OX40, also known as CD134, is a co-stimulatory receptor, of the TNF (tumor necrosis factor) receptor family, expressed on the surface of T cells. Binding of OX40 to its ligand, OX40L (also known as CD252), potentiates T cell activation, differentiation, proliferation, survival and T cell effector function. OX40L is present in NK cells, participating in their activation and cytotoxicity profile, and dendritic cells. OX40 can bind to members of the TRAF (TNFR associated factor) family of proteins, which can then regulate the NF-κF (nuclear factor kappa-light chain enhancer of activated B cells) signaling pathway. OX40 and OX40L can be found in cancer cells, such as AML (acute myeloid leukemia) and breast cancer cells. Studies have shown that OX40 agonists can increase anti-tumor immunity and improve tumor-free survival in pre-clinical studies. Alternatively, OX40 antagonists offer potential as therapeutics for inflammatory diseases. The development of new modulators of the OX40/OX40L activity are promising therapies for patients suffering from solid tumors or auto-immune disorders.

Applications

Screening of small molecules and antibodies that inhibit binding of OX40 to OX40L.

Supplied Materials

Catalog #	Name	Amount	Storage
71185	OX40L (CD252), His-Tag (Human)*	25 µg	-80°C
71310	OX40 (CD134), Biotin-Labeled, His-Tag (Human)*	3 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-OX40 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 **OX40L** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.
2. Dilute OX40L to 4 µg/ml with PBS (50 µl/well, except "Ligand Control" wells).
3. Add 50 µl of diluted OX40L 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 **OX40[biotin]** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.

8. Dilute OX40 to **1 µg/µl** with 1x Immuno Buffer 1 (20 µl/well).

9. Initiate the reaction by adding 20 µl of diluted OX40 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 2 hours 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 OX40 (1 µg/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

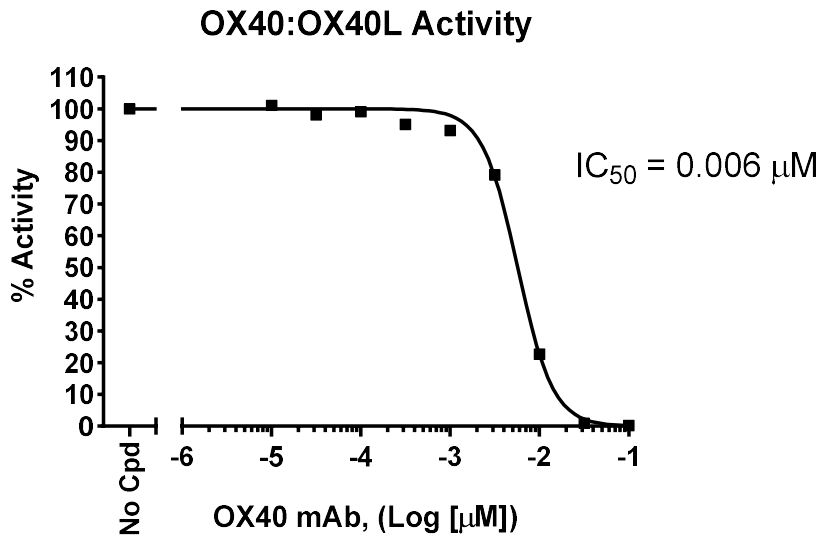

Figure 1: Inhibition of binding of OX40:OX40L by an anti-OX40 antibody.
A plate was coated with OX40L, followed by incubation with OX40 in the presence of increasing concentrations of Anti-OX40 Antibody. Luminescence was measured using a Bio-Tek microplate reader.

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

References

Peng K., *et al.*, 2014 *AAPS J.* 16(4): 625–633.
Marconato M., *et al.*, 2022 *Scientific Reports* 12: 15856.

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*
OX40 Antagonist Antibody	72063	50 µg/100 µg
Anti-OX40 Competitive Antibody	72558	50 µg/100 µg
OX40/NF-κB Reporter – HEK293 Recombinant Cell Line	60482	2 vials
OX40L, His-Avi-Tag Recombinant	100808	100 µg
OX40 (CD134), Fc fusion (Human) Recombinant	71175	100 µg

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