OX40/OX40L Inhibitor Binding Assay Kit

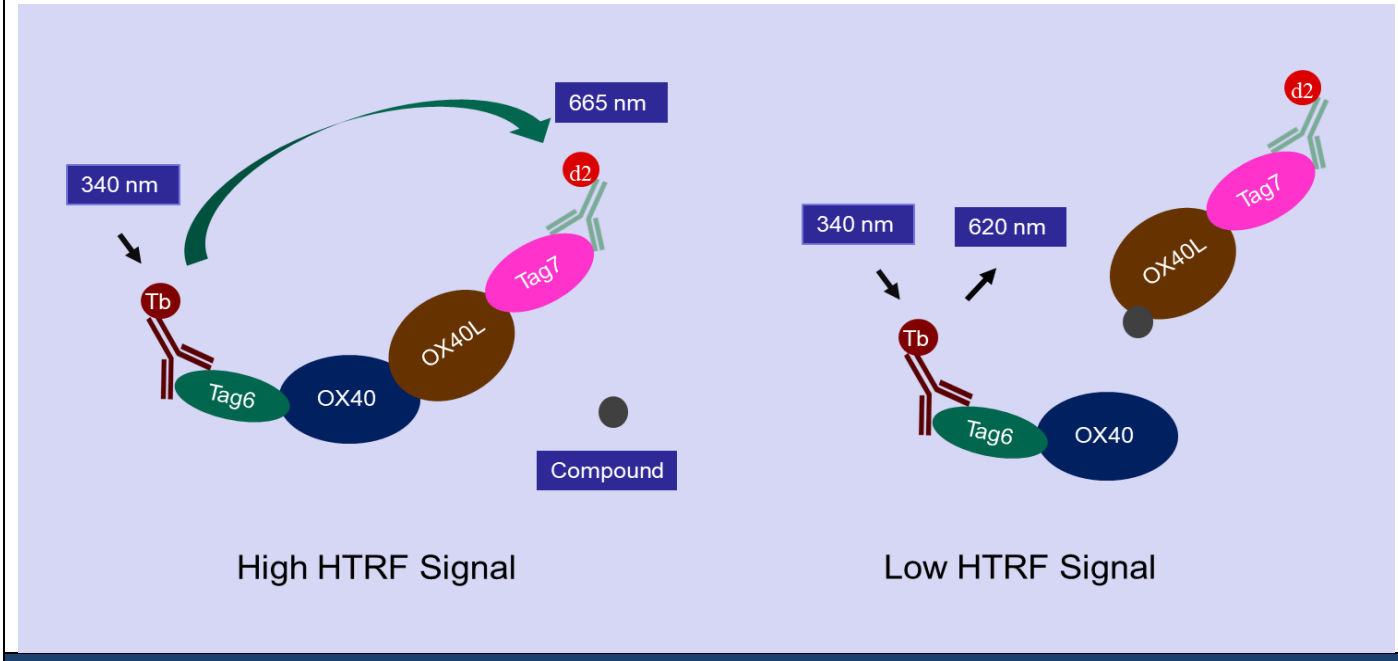
Catalog Number: 2369401

Background

OX40 (CD134; TNFRSF4) is a T-cell co-stimulatory receptor that belong to the tumor necrosis factor receptor superfamily (TNFRSF). Binding of OX40 to OX40L (CD252, CD134L, TNFSF4) triggers signal transduction pathways to activate immune response and regulate T-cell activation, proliferation, differentiation, expansion, and survival. OX40 agonists are being developed and tested in clinical trials for cancer treatment. OX40 antagonists, by inhibiting OX40, can dampen T cell activity, potentially reducing inflammation and autoimmune responses. Modulating the OX40/OX40L pathway have implications for treating inflammatory and autoimmune diseases, as well as cancer immunotherapy.

Assay Principle

Our OX40-OX40L binding assay kit is a TR-FRET based assay designed to detect the binding status between OX40 and OX40L. Tag5-OX40 and Tag7-OX40L are included in this assay kit. Binding of Tag5-OX40 to Tag7-OX40L brings the Terbium (Tb, HTRF donor) and the fluorophore d2 (HTRF acceptor) into proximity, and activation of Tb results in fluorescence resonance energy transfer (FRET). Therefore, the binding status can be quantitively measured by calculating the ratio of the emission fluorescence intensity of the acceptor (665 nm) and donor (620 nm). Disruption of OX40-OX40L binding will reduce the HTRF signal.



Application

High throughput screening of compounds that inhibit the binding between OX40 and OX40L for drug discovery.

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Plate Reader

A HTRF® certified microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) is required.

Components

Catalog number	Item	Amount	Storage
2369402	Assay buffer	25 mL	-20°C
2369403	Recombinant human Tag5-OX40	20 µL	-80°C
2369404	Recombinant human Tag7-OX40L	5 µL	-80°C
728526	Terbium-labeled anti-Tag5 antibody	20 µL	-80°C
432322	fluorescence-labeled anti-Tag7 antibody	20 µL	-80°C
	384-well microplate, White	1	Room temperature

Materials needed but not supplied

1. Microplate reader, HTRF® certified microplate reader
2. 0.5 M DTT
3. Adjustable micro-pipettor
4. Sterile Tips

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Assay protocol

1. Prepare the inhibitor compound solution

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer (since you will add 2 µl to the 20 µl reaction).

   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in 1X assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%). To determine an IC50 or to test lower concentrations of the compound, prepare as series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

2. Prepare OX40 solution

   Thaw OX40 protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted protein at -80°C.

   Note: OX40 protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the OX4L protein 80-fold (1 µL OX40L + 79 µL 1X assay buffer).

   Add 4 µl of diluted protein solution to each well.

3. Add inhibitor

   Add 2 µl of diluted compound solution to each inhibitor test well.

   Add 2 µl of inhibitor solvent solution to each of negative and positive control well.

   Incubate at room temperature for 30 minutes (optional).

4. Prepare OX40L solution

   Thaw OX40 protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted enzyme at -80°C.

   Note: OX40L protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the OX40L protein 600-fold (1 µL OX40L + 599 µL 1X assay buffer).

   Add 4 µl of diluted protein solution to each positive control well and inhibitor test well.

   Add 4 µl of assay buffer to each of negative control well.

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5. Prepare dye solution

   Dilute Terbium-labeled anti-Tag5 antibody and fluorescence-labeled anti-Tag7 antibody 1:200 in assay buffer. For example: 1 µl of Terbium-labeled anti-Tag5 antibody + 1 µl of fluorescence-labeled anti-Tag7 antibody + 198 µl of assay buffer.

   Add 10 µl of this dye mixture to each well.

6. Incubate the reaction at room temperature for 1 hour.
7. Measure fluorescent intensity

   HTRF compatible microplate reader is needed to measure fluorescent intensity of the samples. Fluorescent intensity should be measured twice:

   1. Excitation wavelength at 340 nm and emission at 620 nm.
   2. Excitation wavelength at 340 nm and emission at 665 nm.

Protocol Summary

Component	Negative Control	Positive Control	Inhibitor Test
1X buffer	4 µl		
OX40 protein		4 µl	4 µl
Inhibitor solvent	2 µl	2 µl	
Inhibitor solution			2 µl
Subtotal Volume	**6 µl**	**6 µl**	**6 µl**
Incubate at room temperature for 30 minutes.			
OX40L protein	4 µl	4 µl	4 µl
Dye solution	10 µl	10 µl	10 µl
Total Volume	**20 µl**	**20 µl**	**20 µl**
Incubate at room temperature for 1 hour.			

Data Analysis

1. Calculate the ratio of the fluorescent intensity of each well.

$$Ratio1 = \frac{\text{Fluorescent intensity at 620 nm}}{\text{Fluorescent intensity at 340 nm}}$$

2. Calculate the ratio of the fluorescent intensity of each well.

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$$Ratio2 = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 340 nm}}$$

3. Calculate sample signal.

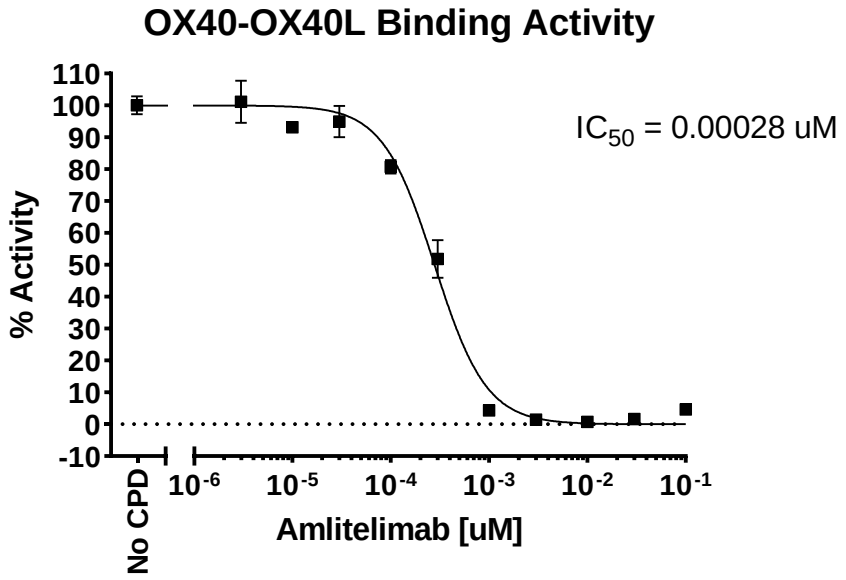
$$Sample\ signal = \frac{Ratio2}{\text{Ratio1}}$$

4. Calculate percentage activity

   In the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\%\ \text{activity} = \frac{S - N}{\text{P} - \text{N}} X100$$

Assay result



Related products:

Product Name	Catalog #	Size
Recombinant Human PD-1	23731	100 µg
Recombinant Human PD-L1	237351	100 µg
Recombinant Human LAG3	235243	100 µg
Recombinant Human FGL1	233451	100 µg

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Recombinant Human CD40	232340	100 µg
Recombinant Human CD40L	2323405	100 µg
Recombinant Human CD27	2323155	100 µg
Recombinant Human CD70	232370	100 µg
Recombinant Human OX40	236940	100 µg
Recombinant Human OX40L	2369405	100 µg
Recombinant Human GITR	234487	100 µg
Recombinant Human GITRL	2344875	100 µg
Recombinant Human CD40	232340	100 µg
Recombinant Human CD40L	2323405	100 µg
Recombinant Human CD155	2323155	100 µg
Recombinant Human TIGIT	2384448	100 µg
SARS-CoV-2 Mpro (3CL Protease) Assay Kit	728203	96 reactions
SARS-CoV-2 Papain-like Protease Assay Kit	728253	96 reactions
SARS-CoV-2 Nucleocapsid Protein Binding Kit (For mouse antibody)	728263	384 reactions
SARS-CoV-2 Nucleocapsid Protein Binding Kit (For rabbit antibody)	728273	384 reactions
DNA Polymerase Theta Activity Assay Kit	362101	96 reactions, 384 reactions
T7 High Yield RNA Synthesis Kit	K777627	25 , 50, 100 reactions
PKMYT1 Binding Assay Kit	756981BK	384 reactions
WEE1 Binding Assay Kit	759331BK	384 reactions
eIF4E/eIF4G Binding Assay Kit	34343BK	384 reactions
Caspase-3 Activity Assay Kit	810030	384 reactions
IDO1 Activity Assay Kit for Inhibitor Screening	910010	96 reactions
Kras WT Nucleotide Exchange Assay Kit	5727-4121NK	384 reactions
Kras G12C Nucleotide Exchange Assay Kit	5727-4122NK	384 reactions
Kras G12D Nucleotide Exchange Assay Kit	5727-4123NK	384 reactions
Kras G13D Nucleotide Exchange Assay Kit	5727-4133NK	384 reactions
Kras G12R Nucleotide Exchange Assay Kit	5727-4127NK	384 reactions
Kras G12V Nucleotide Exchange Assay Kit	5727-4128NK	384 reactions
Kras WT–cRAF Binding Assay Kit	5727-4121BK	384 reactions
Kras G12C–cRAF Binding Assay Kit	5727-4122BK	384 reactions
Kras G12D–cRAF Binding Assay Kit	5727-4123BK	384 reactions
Kras G12R–cRAF Binding Assay Kit	5727-4127BK	384 reactions
Kras G12V–cRAF Binding Assay Kit	5727-4128BK	384 reactions

Products are for research use only and are not intended for human use. We do not sell to patients.

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