Aurora Biolabs
High Purity Protein for Research

PD-1/PD-L1 Inhibitor Binding Assay Kit

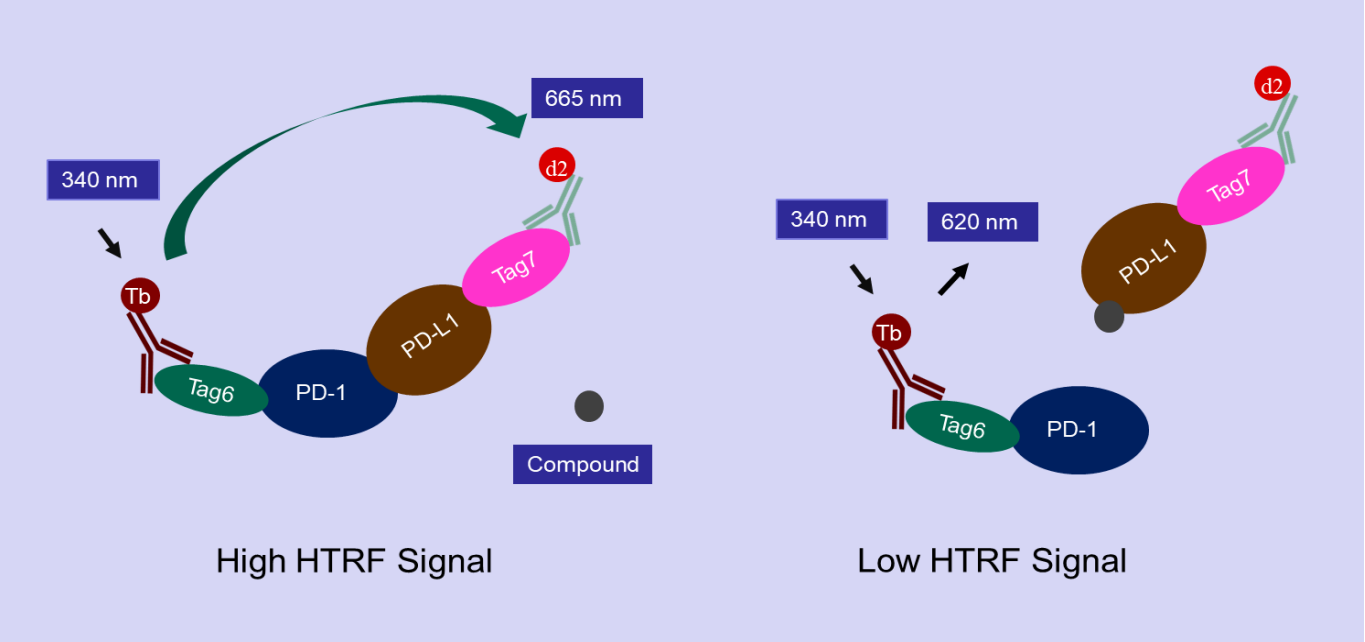
Catalog Number: 237352

Background

Immune checkpoint blockade is a groundbreaking approach in cancer immunotherapy that enhances the immune system's ability to recognize and destroy cancer cells. The immune checkpoint PD-1(CD279)/PD-L1 (CD274 or B7-H1) is an attractive target for cancer immunotherapy. The interaction of PD-1 with PD-L1 induces T cell apoptosis and allows cancer to evade the immune response by suppressing the adaptive immune system. Immunotherapy drugs work by blocking the interaction between PD-1 and PD-L1, and enhancing the anti-tumor immune response.

Assay Principle

The PD-1/PD-L1 binding assay kit is a TR-FRET based assay, which is designed to detect the binding status between PD-1 and PD-L1. Tag6-PD-1 and Tag7-PD-L1 are included in this assay kit. Binding of Tag6-PD-1 to Tag7-PD-L1 brings the Terbium (Tb, HTRF donor) and the fluorophore d2 (HTRF acceptor) in a proximity distance, and activation of Tb results in fluorescence resonance energy transfer (FRET). Thus, 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). Interference of the PD-1/PD-L1 binding will reduce the HTRF signal.



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Application

High throughput screening of compounds that inhibit the binding between PD-1 and PD-L1 for drug discovery.

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
234822-B	Assay buffer	25 mL	-20°C
237353	Recombinant human Tag6-PD-1	60 µL	-80°C
237354	Recombinant human Tag7-PD-1L	5 µL	-80°C
728526	Terbium-labeled anti-Tag6 antibody	20 µL	-80°C
432322	fluorescence-labeled anti-Tag7 antibody	40 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 PD-1 solution

   Thaw PD-1 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: PD-1 protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the PD-1 protein 32-fold (1 µL PD-1 + 31 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 PD-L1 solution

   Thaw PD-L1 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: PD-L1 protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the PD-L1 protein 400-fold (1 µL PD-L1 + 399 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.

5. Prepare dye solution

   Dilute Terbium-labeled anti-Tag6 antibody and fluorescence-labeled anti-Tag7 antibody 1:200 in assay buffer. For example: 1 µl of Terbium-labeled anti-Tag6 antibody + 2 µl of fluorescence-labeled anti-Tag7 antibody + 197 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		
PD-1 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.			
PD-L1 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 HTRF signal of each well.

$$HTRF\ signal = \frac{\text{Fluorescent intensity at 620 nm}}{\text{Fluorescent intensity at 650 nm}} X10{,}000$$

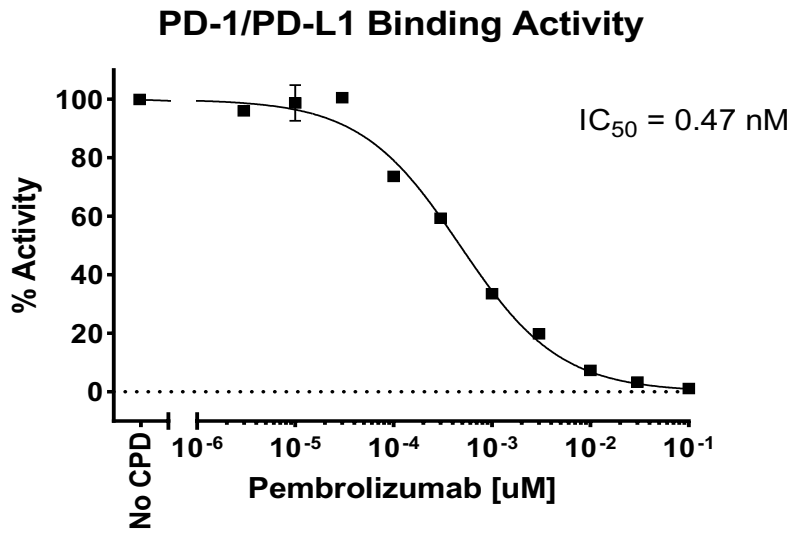
2. Calculate percentage activity

   In the absence of the compound (positive control), the sample HTRF signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample HTRF 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 HTRF signal in the presence of the compound.

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

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
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

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TEV Protease	190001	1,000 Units, 10,000 Units
TEV Protease- His-tag	190001-R	50 ug, 200 ug, 1 mg
PreScission Protease (HRV 3C)	190002	1,000 units, 10,000 units
Recombinant SUMO Protease (Ulp1)	190003	1,000 units, 10,000 units
Recombinant YopH	200100	10 ug, 20 ug, 100 ug, 1 mg
Recombinant Biotin Protein Ligase (BirA)	90101	100 ug
Recombinant SortaseA-5M	90201	50 ug, 200ug
Recombinant Mouse Leukemia Inhibitory Factor	11-0001	10 ug, 100 ug
Recombinant Human LIF	12-0002	10 ug, 100 ug, 1 mg
Recombinant Human FGF-basic, Carrier-free	12-0005CFR	50 ug, 100 ug, 500 ug, 1 mg
Human SOS1, Avi-His tag	7671HA	50 µg, 100 µg
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
Kras G13D–cRAF Binding Assay Kit	5727-4133BK	384 reactions
Kras WT/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4121CK	384 reactions
Kras G12C/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4122CK	384 reactions
Kras G12D/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4123CK	384 reactions
Kras G12V/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4128CK	384 reactions
Kras G13D/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4133CK	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
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
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