BIS09-CN.01

Bispecific Human PD-L1 & VEGFA Bridging ELISA Kit

Pack Size: 96 tests

Catalog Number: BIS-A009

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

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【Intended Use】

Bispecific Human PD-L1 & VEGFA Bridging ELISA Kit is intended for the detection of bispecific anti-PD-L1 & VEGFA antibodies in purified recombinant proteins, human serum, and cell culture supernatants. It is intended for research use only (RUO).

【Background】

Bispecific antibodies (BsAbs) are engineered immunoglobulins that simultaneously bind two distinct antigens or two different epitopes on the same antigen. Their function depends on precise heavy- and light-chain pairing and molecular architecture, which determine avidity, orientation, and functional potency. By physically bridging immune effector cells to target cells, BsAbs can redirect and amplify immune-mediated cytotoxicity, offering enhanced tumor killing and a reduced likelihood of resistance compared with some monospecific therapies.

VEGFA is a major member of the vascular endothelial growth factor (VEGF)/platelet-derived growth factor (PDGF) family and plays a critical role in angiogenesis and endothelial cell function. During the differentiation of human pluripotent stem cells (hPSCs) into endothelial cells, VEGFA promotes the commitment of mesodermal cells to endothelial progenitors and stimulates endothelial cell proliferation, migration, and tube formation. Programmed cell death 1 ligand 1 (PD-L1) is a member of the B7 family of immune regulatory molecules and plays an important role in regulating cellular and humoral immune responses. This protein is widely expressed in peripheral tissues and hematopoietic cells. PD-L1 inhibits T-cell responses by suppressing T-cell activation and proliferation and by inducing T-cell dysfunction. A PD-L1×VEGFA bridging ELISA evaluates a bispecific molecule’s ability to form the ternary complex between PD-L1 and VEGFA, providing a high-throughput, mechanism-relevant readout of dual engagement that informs potency, lead selection, and quality control.

【Assay Principle】

This kit is used to measure the levels of anti-PD-L1 & VEGFA antibodies using a Bridging-ELISA format. The microplate is pre-coated with a Human VEGFA Protein, which captures anti-PD-L1 & VEGFA antibodies present in standards and samples, then a Biotin-Human PD-L1 Protein is added to bind the captured anti-PD-L1 & VEGFA antibodies, forming an antigen-antibody-biotinylated antigen complex. After washing, a Streptavidin-HRP is added to the plate. Following additional washes, a substrate is added for color development. The reaction is stopped with stop solution, and the color changes from blue to yellow. Absorbance is measured at 450 nm with a 630 nm reference.

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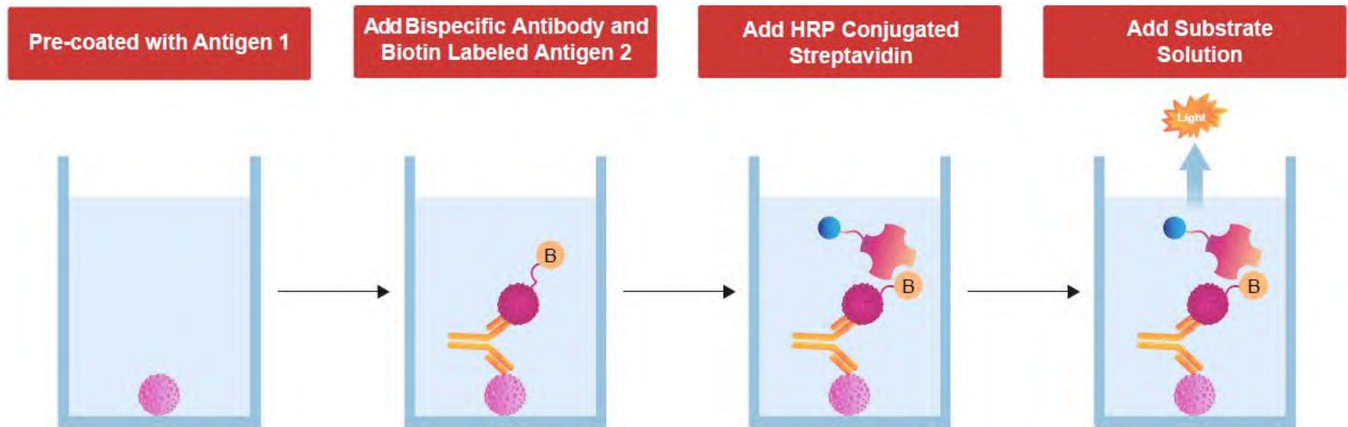
The absorbance signal is directly proportional to the amount of anti-PD-L1 & VEGFA antibodies in the sample.

Figure 1. Bispecific Bridging ELISA Assay Principle



【Materials Provided】

Table 1. Materials Provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
BIS009-C01	Pre-coated Human VEGFA Protein Microplate	1 plate	Solid	2-8℃	2-8℃
BIS009-C02	Anti-PD-L1 & VEGF Antibody Standard	20 μg	Powder	2-8℃	-70℃
BIS009-C03	Biotin-Human PD-L1 Protein	20 μg	Powder	2-8℃	-70℃
BIS009-C04	Streptavidin-HRP	20 μg	Powder	2-8℃, avoid light	-70℃, avoid light
BIS009-C05	10×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
BIS009-C06	2×Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
BIS009-C07	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
BIS009-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

【Reagents and Consumables / Equipment Required but not Provided】

Single-or dual-wavelength microplate reader with 450nm and 630nm filters;

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Incubator;

Single- or multi-channel micropipettes;

10 μL, 200 μL, and 1000 μL precision pipettes;

Centrifuge tubes;

Timer;

Reagent bottle;

Deionized water or ultrapure water;

【Storage】

1. Store the unopened kit at 2-8°C upon receipt.
2. Locate the expiration date on the outer packaging and do not use reagents beyond their expiration date.
3. The opened kit should be stored according to the storage conditions listed in the Components Table. The shelf life of the opened kit is 30 days from the date of opening.

【Reagent Preparation】

Bring all reagents to room temperature (20-25°C) before use. If crystals are present in the solution, allow the reagents to equilibrate until the crystals are completely dissolved. If needed, incubate at 37°C for 10-15 minutes to facilitate dissolution.

According to Table 3, reconstitute the provided lyophilized product with ultrapure water to prepare the stock solution. Allow the vial to stand at room temperature for 15 to 30 minutes, then gently pipette up and down to mix. Do not vortex or shake vigorously.

Store the reconstituted stock solution at -70°C. It is recommended to aliquot the stock solution to avoid repeated freeze-thaw cycles. Do not exceed one freeze-thaw cycle. Each aliquot should contain at least 5 μg of material.

Table 2. Preparation Method

ID	Components	Size	Stock Solution Conc.	Reconstitution Buffer and Vol.
BIS009-C02	Anti-PD-L1 & VEGF Antibody Standard	20 μg	200 μg/mL	100 μL water
BIS009-C03	Biotin-Human PD-L1 Protein	20 μg	200 μg/mL	100 μL water
BIS009-C04	Streptavidin-HRP	20 μg	100 μg/mL	200 μL water

【Assay Procedure】

1. Preparation of Working Solution

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1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to a final volume of 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to a final volume of 100 mL.

1.3 Preparation of Biotin-Human PD-L1 Protein working solution:

Dilute Biotin-Human PD-L1 Protein to 0.4 μg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working solution:

Dilute Streptavidin-HRP to 0.1 μg/mL with 1×Dilution Buffer. The prepared working solution should avoid light. Please prepare it for one-time use only.

1.5 Sample preparation:

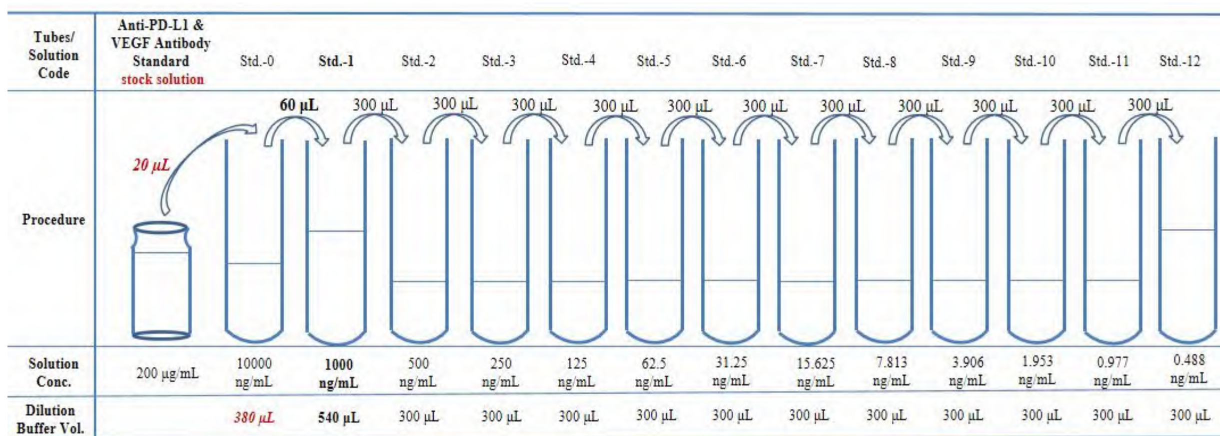
a. If the test sample is cell culture supernatant, dilute the sample at a 1:2 dilution ratio using 1× Dilution Buffer (sample: diluent = 1:1, v/v).

b. If the test sample is serum, dilute the sample at a 1:2 dilution ratio using 1× Dilution Buffer (sample: diluent = 1:1, v/v).

2. Preparation of Standard

Prepare serial dilutions of the Anti-PD-L1 & VEGF Antibody Standard using 1×Dilution Buffer as recommended in Figure 2.

Figure 2. Preparation of Anti-PD-L1 & VEGF Antibody Standard



3. Addition of Samples

Add **50 μL** of serially diluted **Anti-PD-L1 & VEGF Antibody Standard** and samples to the corresponding wells. Add **50 μL** of 1×Dilution Buffer to the Blank control wells. Then add **50 μL of Biotin-Human PD-L1 Protein (diluted to 0.4 μg/mL)** working solution to each well. Shake the

plate gently for 5s to ensure thorough mixing. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

Note: a. It is recommended to run all standards and samples in duplicate wells.

b. Due to the addition method of 50 μL+50 μL, the final concentration in the standard curve well differs by two times from the dilution concentration.

4. Washing

Remove the remaining solution by aspiration, add 300 μL of 1×Washing Buffer to each well, soak for 30s, remove any remaining 1×Washing Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the washing step above three times.

5. Addition of Streptavidin-HRP

Add 100 μL **Streptavidin-HRP (dilute to 0.1 μg/mL)** working solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for **30 minutes**.

6. Washing

Repeat step 4.

7. Substrate Reaction

Add 100 μL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at room temperature for **15 minutes**, avoid light.

8. Termination

Add 50 μL **Stop Solution** to each well. Gently tap the plate to ensure thorough mixing.

Note: The color in the wells will change from blue to yellow.

9. Data Recording

Measure the absorbance at 450 nm with a 630 nm reference within 5 minutes after adding the stop solution.

Note: Subtracting the OD_{630nm} value from the OD_{450nm} value helps reduce background interference.

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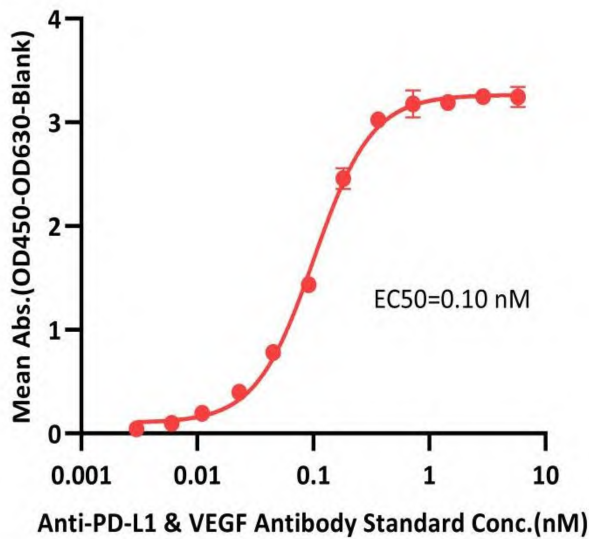
【Typical Data】

Absolute OD values may vary depending on the laboratory, operator, and equipment used. The example data provided below is for reference only.



Anti-PD-L1 & VEGF Antibody Standard (ng/mL)	Anti-PD-L1 & VEGF Antibody Standard (nM)	Mean Abs(OD450-630nm)	Mean Abs(OD450-630nm-Blank)
1000	5.800	3.282	3.247
500	2.900	3.282	3.247
250	1.450	3.228	3.193
125	0.725	3.213	3.178
62.5	0.363	3.059	3.024
31.25	0.181	2.493	2.458
15.625	0.091	1.469	1.434
7.813	0.045	0.816	0.781
3.906	0.023	0.432	0.397
1.953	0.011	0.231	0.196
0.977	0.006	0.133	0.098
0.488	0.003	0.079	0.044
Blank	Blank	0.035	0.000

【Precautions】

【Troubleshooting Guide】

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells

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High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper washing * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again

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