BIS08-EN.01

Bispecific Human PD-1 & VEGFA Bridging ELISA Kit

Pack Size: 96 tests

Catalog Number: BIS-A008

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

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure

INTENDED USE

Bispecific Human PD-1 & VEGFA Bridging ELISA Kit is developed for the detection of anti-PD-1 & VEGFA antibodies in serum and cell culture supernates. 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.

VEGF165 is the most abundant splice variant of VEGF-A. VEGF165 is produced by a number of cells including endothelial cells, macrophages and T cells. VEGF165 is involved in angiogenesis, vascular endothelial cell survival, growth, migration and vascular permeability. VEGF gene expression is induced by hypoxia, inflammatory cytokines and oncogenes. VEGF165 binds to heparan sulfate and is retained on the cell surface and in the extracellular matrix. VEGF plays a key role in tumor angiogenesis in many cancers. Programmed cell death protein 1 (PD-1) is also known as CD279 and PDCD1, is a type I membrane protein and is a member of the extended CD28/CTLA-4 family of T cell regulators. PDCD1 is expressed on the surface of activated T cells, B cells, macrophages, myeloid cells and a subset of thymocytes. A PD-1 & VEGFA bridging ELISA evaluates a bispecific molecule's ability to form the ternary complex between PD-1 and VEGFA, providing a direct measure of the antibody's primary mechanism of action.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of anti-PD-1 & VEGFA antibodies by employing a standard Bridging-ELISA format. The microplate in the kit has been pre-coated with Human VEGFA Protein. First add the standard samples provided in the kit and your samples to the plate, then add the Biotin-Human PD-1 Protein to the plate, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. Lastly load the substrate into the wells and monitor color development in proportion with the amount of anti-PD-1 & VEGFA

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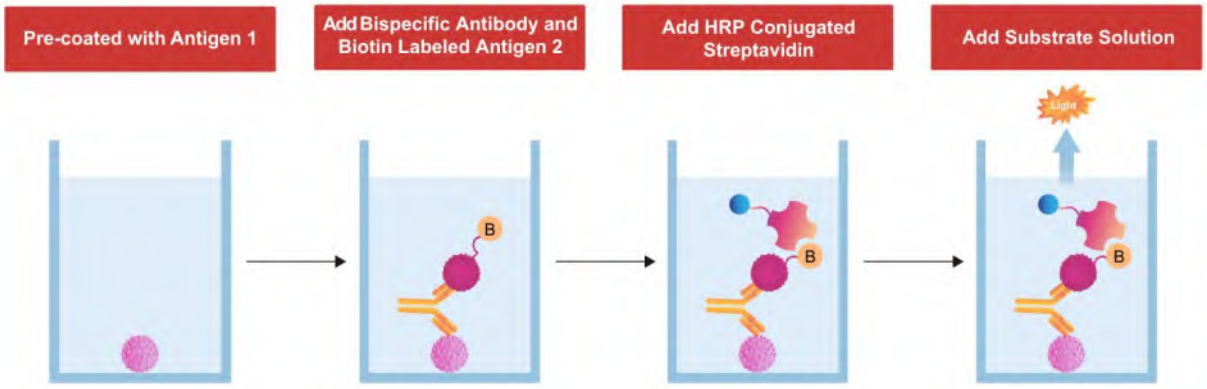
antibodies present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of anti-PD-1 & VEGFA antibodies bound.

FIGURE 1. Bispecific Bridging ELISA Assay Principle



MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
BIS008-C01	Pre-coated Human VEGFA Protein Microplate	1 plate	Solid	2-8°C	2-8°C
BIS008-C02	Anti-PD-1 & VEGF Antibody Standard	20 μg	Powder	2-8°C	-70°C
BIS008-C03	Biotin-Human PD-1 Protein	20 μg	Powder	2-8°C	-70°C
BIS008-C04	Streptavidin-HRP	20 μg	Powder	2-8°C, avoid light	-70°C, avoid light
BIS008-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
BIS008-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
BIS008-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
BIS008-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

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REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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

Centrifuge;

Incubator;

10 µL, 200 µL and 1000 µL precision pipettes;

10 µL, 200 µL and 1000 µL pipette tips;

Multichannel pipettes;

Tubes;

Graduated cylinder to prepare Wash Solution;

Deionized or distilled water to dilute 10×Washing Buffer;

STORAGE AND VALIDITY INSTRUCTIONS

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

1. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in a 37 ℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.
2. Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table 2 and place the materials for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking. The reconstituted stock solutions should be stored at -70℃. It is recommended not to freeze-thaw more than 1 time, the packing specification shall not be less than 5µg.

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TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
BIS008-C02	Anti-PD-1 & VEGF Antibody Standard	20 μg	200 μg/mL	100 μL water
BIS008-C03	Biotin-Human PD-1 Protein	20 μg	200 μg/mL	100 μL water
BIS008-C04	Streptavidin-HRP	20 μg	100 μg/mL	200 μL water

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Human PD-1 Protein working fluid:

Dilute Biotin-Human PD-1 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 fluid:

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

1.5 Sample preparation:

a. If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:1.

b. If the sample to be tested is serum, dilute test sample at 1:10 with 1×Dilution Buffer. The volume ratio of samples to diluent is 1:9.

2. Preparation of Standard curve

Make serial dilutions of the Anti-PD-1 & VEGF Antibody as a Standard curve with Dilution Buffer as recommended in Figure 2.

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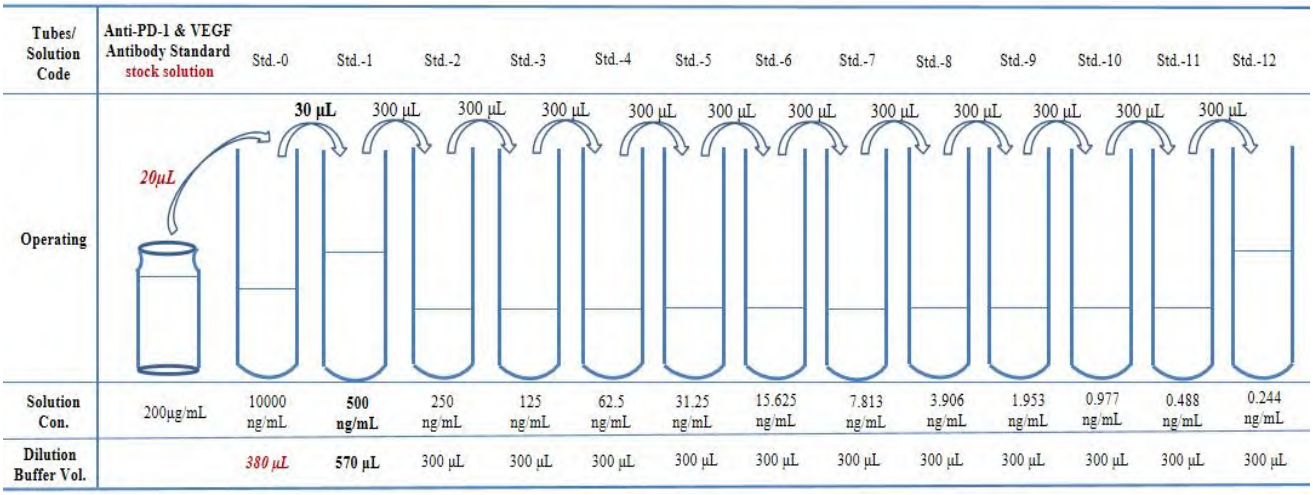
FIGURE 2. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Anti-PD-1 & VEGF Antibody



3. Add samples

Add **50 µL** serially diluted **Anti-PD-1 & VEGF Antibody Standard** curve and samples to each well. For blank Control wells, please add **50 µL 1×Dilution Buffer**. Then add **50 µL Biotin-Human PD-1 Protein (dilute to 0.4 µg/mL)** working fluid to each well. Shake gently for 5s to mix. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

Note: a. It is recommended to set double holes for samples and standard curves to be tested.

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 30 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

5. Add Streptavidin-HRP

For all wells, add 100 µL **Streptavidin-HRP (dilute to 0.1 µg/mL)** working solution. Seal the plate with microplate sealing film and incubate at room temperature for **30 min**.

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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 min**, avoid light.

8. Termination

Add 50 µL **Stop Solution** to each well and tap the plate gently to allow thorough mixing.

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

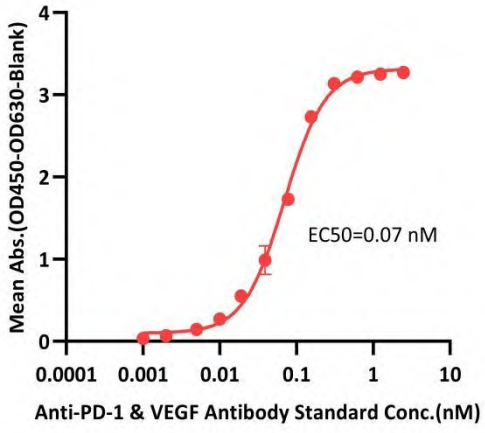
9. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 5 minutes.

Note*: To reduce the background noise, subtract the value read at* $OD_{450\,nm}$ *with the value read at* $OD_{630\,nm}$.

TYPICAL DATA

The following data is for reference only, and the specific OD value may vary depending on different laboratories, experimenters, or equipments.



Anti-PD-1 & VEGF Antibody Standard (ng/mL)	Anti-PD-1 & VEGF Antibody Standard (nM)	Mean Abs(OD450-630nm)	Mean Abs(OD450-630nm-Blank)
500	2.488	3.297	3.261
250	1.244	3.218	3.182
125	0.622	3.320	3.284
62.5	0.311	3.222	3.186
31.25	0.155	2.767	2.731
15.625	0.078	1.765	1.729
7.813	0.039	1.025	0.989
3.906	0.019	0.586	0.550
1.953	0.010	0.309	0.273
0.977	0.005	0.182	0.146
0.488	0.002	0.105	0.069
0.244	0.001	0.070	0.034
Blank	Blank	0.036	0.000

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PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. All reagents should be balance to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.
5. The kit should be stored at 2°C-8°C.

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
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * 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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