Human PD1/PDL1 Inhibitor Screening Assay Kit

Catalogue No.: EH4514

Revision: V3.2

Size: 48T/96T

Reactivity: Human

Range: 0.313-20ng/ml

Sensitivity: 0.188ng/ml

Application: For screening and profiling inhibitors of PD1/PDL1

Storage: 2-8°C

Expiry Date: see kit label

Principle: Competitive

NOTE: FOR RESEARCH USE ONLY.

Kit Components

No.	Item	Specifications(48T/96T)	Storage
E001	ELISA Microplate(Dismountable)	8×6/8×12	2-8°C/-20°C
E002	Assay Control (10ng/vial, Lyophilized)	5vial/10vial	2-8°C/-20°C
E004	Positive Control	1ml/1ml	2-8°C
E039	Sample Dilution Buffer	10ml/20ml	2-8°C
E003	Biotin-labeled Antibody(Concentrated)	60ul/120ul	2-8°C(Avoid Direct Light)
E040	Antibody Dilution Buffer	5ml/10ml	2-8°C
E034	HRP-Streptavidin Conjugate(SABC)	60ul/120ul	2-8°C(Avoid Direct Light)
E049	SABC Dilution Buffer	5ml/10ml	2-8°C
E024	TMB Substrate	5ml/10ml	2-8°C(Avoid Direct Light)
E026	Stop Solution	5ml/10ml	2-8°C
E038	Wash Buffer(25X)	15ml/30ml	2-8°C
E006	Plate Sealer	3/5pieces	
E007	Product Description	1copy	

Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

Standard(n=5)	37°C for 1 month	2-8°C for 6 months
Average (%)	80	95-100

To minimize extra influence on performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is strongly suggested that the same operator performs the whole assay from the beginning to the end.

Operation Procedure

Principle of the Assay

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with PD-L1 protein. Human PD-1 Protein is used as an analytical Assay Control. During the reaction, Inhibitor in the sample Inhibit PD1 and PDL1 binding. Excess unbound sample or standard are washed from the plate, and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP -Streptavidin was added and unbound conjugates were washed away with wash buffer. Then TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm. The inhibition ratio of Inhibitor in the samples is then determined by comparing the OD of the samples to the standard.

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required but Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Washing

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute. (**Note**: set the height of the needles; be sure the fluid can be sipped up completely)

Sample Dilution

The user should estimate the inhibitor concentration, in the test sample, and select a proper dilution factor. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

Reagent Preparation and Storage

Bring all reagents and samples to room temperature for 20 minutes before use.

1, Wash Buffer:

If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

Dilute 30ml (15ml for 48T) Concentrated Wash Buffer to 750ml (375ml for 48T) Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

2, Preparation of Assay Control and Sample

1). Add 500ul Sample Dilution Buffer into Assay Control tube, keep the tube at room temperature for 1-2 minutes and mix them thoroughly.

2). Diluted sample with sample dilution buffer

Note: Store the dissolved Assay Control (20ng/ml) at 2-8°C and use it within 12h.

3, Preparation of Biotin-labeled Antibody Working Solution:

Prepare it within 1 hour before experiment.

1) Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

2) Dilute the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul Biotin-labeled antibody into 99ul Antibody Dilution Buffer.)

4, Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

Prepare it within 30 minutes before experiment.

1) Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

2) Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul of SABC into 99ul of SABC Dilution Buffer.)

Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. Set Positive Control, Assay control, test samples, blank wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each control and sample in duplicate. **Wash plate 2 times before adding Positive Control, Assay control, sample and control (blank) wells!**

2. Solutions are added to the bottom of microplate well, avoiding inside wall touching and foaming as much as you can.

	Blank well	Positive Control well	Assay control well	Sample well
Assay Control (PD1 protein,20ng/ml)	-	50ul	50ul	50ul
Positive Control	-	50ul	-	-
Sample Dilution Buffer	100ul	-	50ul	-
diluted sample	-	-		50ul

3. Cover with the Plate sealer we provided. Gently tap the plate for 1 min to ensure thorough mixing. Incubate for 90 minutes at 37°C.

4. **Wash:** Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.

5. **Biotin-labeled Antibody:** Add 100ul Biotin-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 60 minutes.

6. **Wash:** Remove the cover, and wash plate 3 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 minutes each time.

7. **HRP-Streptavidin Conjugate (SABC):** Add 100ul of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.

8. **Wash:** Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.

9. **TMB Substrate:** Add 90ul TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (**Note:** The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes.)

10. **Stop:** Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.

11. **OD Measurement:** Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

Test data:

Test group	Assay Control(10ng/ml PD-1 Protein)	Positive control	Sample	Blank
Number	A	B	C	D
OD450	1.8-2.4	0.3-0.6	0.1-2.4	0.1-0.25

12. **Data calculation:** Inhibition rate (%)=OD450[(A-C)/A]×100

Summary

Step1: Wash plate 2 times before adding Positive Control, Assay Control, Sample and blank wells!

Step2: Add sample or inhibitors, Positive Control and blank into each well, gently tap the plate for 1 min to ensure thorough mixing then incubate for 90 minutes at 37°C.

Wash step: Aspirate and wash plates 2 times.

Step2: Add 100ul Biotin-labeled antibody working solution to each well and incubate for 60 minutes at 37°C.

Wash step: Aspirate and wash plates 3 times.

Step3: Add 100ul SABC Working Solution into each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 5 times.

Step4: Add 90ul TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

Step6: Add 50ul Stop Solution. Read at 450nm immediately and calculation.