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.

Human PD1/PDL1 Inhibitor Screening Assay Kit

货号: EH4514

规格: 48T/96T

适用种属: Human

检测范围: 0.313-20ng/ml

敏感性: 0.188ng/ml

应用: 用于筛选和分析 PD1/PDL1 抑制剂。

储存条件:2-8°C

保质期: 见试剂盒标签

实验方法: 竞争法

注: 仅供科研使用

试剂盒组件:

No.	Item	Specifications(48T/96T)	Storage
E001	ELISA Microplate(Dismountable)	8×6/8×12	2-8°C/-20°C
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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	

精密度

批内差: CV<8%

批间差: CV<10%

稳定性

ELISA 试剂盒的稳定性取决于活性丧失率。在适当的存储条件下，该试剂盒在有效期内的活性丧失率小于 10%。

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

为了最大程度地减少操作和实验室条件（尤其是空气温度，湿度）对试剂盒的效价影响，应严格控制恒温箱温度。而且强烈建议由同一操作员从头到尾操作整个实验。

操作步骤

实验原理

该试剂盒基于竞争 ELISA 检测法。试剂盒中提供的微孔板已预先包被 PD-L1 蛋白。人 PD-1 蛋白作为显色质控品（Assay Control）。人 PD-1 蛋白与结合在酶标板上的 PD-L1 蛋白结合。反应时，样品中的抑制剂抑制 PD1 和 PDL1 的结合。从板上洗去未结合的样品或显色质控品。将生物素偶联的抗 PD-1 的检测抗体加入孔中，孵育完成后用洗涤缓冲液洗涤板孔。然后加入 HRP-链霉亲和素(SABC)，反应后用洗液洗去未结合的偶联物。再将 TMB 底物溶液添加到每个孔中进行显色（蓝色）。通过添加硫酸溶液终止酶-底物反应，并通过分光光度法在 450nm 的波长处测量 OD 值。然后通过计算抑制比率来衡量样品对 PD1/PDL1 的抑制作用。

注意事项

11. 为了保证实验操作的有效性和样品稀释比例的适当性，建议使用标准品和少量样品进行预实验。
12. 打开后和使用前，请保持微孔板干燥。暂不使用的酶标板孔请加干燥剂后密封保存，避免受潮。
13. 在使用试剂盒之前，使用离心机旋转试管，将试剂离心至试管底部。
14. TMB 试剂应避光存放。
15. 洗涤过程很重要，不充分的洗涤容易造成假阳性和高背景。
16. 对于待测样品的测量反应，建议使用双孔测定，减小实验误差。
17. 检测过程中，请提前准备好下一步实验所需试剂，洗板后及时将试剂加入板孔，不要让微孔板过分干燥，因为干燥的孔板会使板上的活性成分失活。
18. 请勿重复使用枪头，以免造成交叉污染。
19. 本公司不同试剂盒内的试剂，成分不同，请勿混用。不可混用其他厂家的试剂。
20. 为了确保结果的准确性，加样完成，贴封板膜以防止孵育过程样品的蒸发，然后在推荐温度下完成孵育过程。

试剂盒组件外所需器材和试剂

7. 酶标仪（波长：450nm）

8. 37°C 恒温箱
9. 自动洗板机
10. 精密的单道和多道移液器以及干净的一次性枪头
11. 干净的 EP 管
12. 去离子水或蒸馏水

洗板方法

手动洗板：将微孔板中的溶液倒出，在吸收性滤纸或其他吸收性材料上中度力拍打。每孔中加入 350ul 洗液，并浸泡 1 至 2 分钟后，将洗液从板中倒出，在吸收性滤纸或其他吸收性材料上中度力拍打。

自动洗板：用自动洗板机洗板，设定洗板次数，浸泡时间及每孔洗液加样量。每孔的洗液加液量不少于 350ul。最后一次洗板后，翻转微孔板，在吸收性滤纸或其他吸收性材料上中度力拍打除去残留洗液。建议将洗板机设置为浸泡 1 分钟。（注意：设置自动洗板机的针高度，以确保可以完全吸出液体）

样品稀释

用户应估计测试样品中的抑制剂浓度，并选择合适的稀释因子。用提供的稀释缓冲液稀释样品，可能需要进行几次试验。测试样品必须与稀释缓冲液充分混合。如果样品浓度非常高，请先用 PBS 稀释样品，然后用样品稀释稀释样品。

试剂准备与储存

使用前，将所有试剂和样品置于室温 20 分钟。

1, 洗涤缓冲液:

如果浓缩的洗涤液中形成了晶体，可以在 40°C 水浴中加热（加热温度不应超过 50°C），轻轻混合直至晶体完全溶解。

用去离子水或蒸馏水（推荐电阻率为 18MΩ 的超纯水）将 30ml 浓缩洗涤液（48T 为 15ml）稀释至 750ml（48T 为 375ml）并混匀。或依实验所需，取适量浓缩洗涤液稀释至 25 倍体积并混匀，将未使用的溶液放回 2-8°C。

2, Assay Control 和样品的准备:

1）. 将 500ul 样品稀释缓冲液加入一个 Assay Control 管中，将管在室温下保持 1-2 分钟并充分混合。

2）. 用样品稀释缓冲液适度稀释样品。

注: 稀释后的 Assay Control 管(20ng/ml)请保存于 2-8°C，并于 12 小时内使用完。

3, 生物素标记抗体工作液的制备:

1) 计算所需的工作溶液总体积：100ul/孔 X 孔的数量。（最好准备比总体积多 0.1-0.2ml 的量。）
2) 用抗体稀释缓冲液，按 1：100 的比例稀释生物素标记的抗体，并将其充分混合。（即将 1ul 生物素标记的抗体添加到 99ul 抗体稀释缓冲液中。）

4, HRP-链霉亲和素偶联物（SABC）工作液的制备：

实验前 30 分钟内准备。

1）计算所需的工作溶液总体积：0.1ml/孔 X 孔的数量。（最好准备比总体积多 0.1-0.2ml 的量。）

2）用 SABC 稀释液按 1：100 的比例稀释 SABC，并充分混合。（即将 1ul SABC 加入 99ul SABC 稀释液中。）

测定步骤

稀释样品和试剂时，必须将它们完全均匀地混合。TMB 加入孔中之前，请将 TMB 底物在 37°C 下平衡 30 分钟。

13. 在酶标板上设置阳性对照，Assay Control，测试样品，空白孔，然后记录其位置。建议一式两份测量每个对照品和样品。**在加入阳性对照，Assay Control，待测样品和对照（空白）孔之前清洗板 2 次！**

14. **加样：**根据下表添加样品。加样完成后，贴上覆膜，轻轻震荡酶标板 1 分钟，以确保充分混合。在 37° C 下孵育 90 分钟。（将溶液添加到微孔板孔的底部，尽可能避免接触内壁和起泡。）

	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

15. **洗板:** 取下覆膜，并用洗涤缓冲液洗板 2 次，每次浸泡 1 分钟。最后一次洗涤后，通过抽吸或倾倒除去所有的洗涤缓冲液。

16. **加生物素标记抗体工作液:**每孔加 100ul 生物素标记抗体工作液。盖上新的覆膜，37°C 孵育 60 分钟。

17. **洗板:** 取下覆膜，并用洗涤缓冲液洗板 3 次，每次浸泡 1 分钟。最后一次洗涤后，通过抽吸或倾倒除去所有的洗涤缓冲液。

18. **加 HRP-链霉亲和素结合物（SABC）:** 每孔加 100ul SABC 工作液。盖上新的覆膜，37°C 孵育 30 分钟。

19. **洗板:** 取下覆膜或盖板，用洗涤缓冲液洗涤板 5 次，每次浸泡 1-2 分钟。最后一次洗涤后，通过抽吸或倾倒除去所有的洗涤缓冲液。

20. **加 TMB 底物溶液:** 每孔加入 90ul TMB 底物溶液，加覆膜，在 37°C 下于暗箱中孵育 10-20 分钟。**（在将 TMB 加入孔中之前，请将 TMB 底物在 37°C 下平衡 30 分钟）**（注意：根据颜色的实际变化，反应时间可以缩短或延长，但不能超过 30 分钟。当 Assay Control 孔中出现明显的较深蓝色时，可以终止反应。）

21. **加终止液:** 向每孔中添加 50ul 终止液。颜色将由蓝色立即变为黄色。添加终止液的操作顺序与添加 TMB 底物溶液的操作顺序相同。

22. **OD 值的测量:** 加入终止溶液后，立即在酶标仪的 450nm 吸光处进行吸光度测定，读取 OD450 数值。

测试数据:

Test group	Assay Control(20ng/ml PD-1 Protein)	阳性对照	样品	空白孔
编号	A	B	C	D
OD450	1.8-2.4	0.3-0.6	0.1-2.4	0.1-0.25

11，结果结算：抑制比率 (%)=OD450[(A-C)/A]×100

总结

步骤 1: **在加入阳性对照，Assay Control，待测样品和对照（空白）孔之前清洗板 2 次！**

步骤 1: 将样品或抑制剂、阳性对照、Assay Control 及样品稀释液按表格所示加入对应的孔中，轻轻震荡酶标板 1 分钟，以确保充分混合，然后在 37° C 下孵育 90 分钟。

洗板:洗板 2 次。

步骤 2:向每个孔中加入 100ul 生物素标记抗体工作液，覆膜，并在 37°C 下孵育 60 分钟。

洗板:洗板 3 次。

步骤 3:向每个孔中加入 100ul HRP -链霉亲和素偶联物(SABC)工作液，覆膜，并在 37°C 下孵育 30 分钟。

洗板:洗板 5 次。

步骤 4:添加 90ul TMB 底物溶液。覆膜，并在 37°C 下孵育 10-20 分钟。

步骤 5:添加 50ul 终止液。立即在 450nm 处读取 OD450 值并计算。