Primacu™ ELISA

Human Programmed cell death 1 ligand 1 ELISA Kit

User instruction

Cat. No. BPE016

Standard Curve Range: 12.5-800 pg/mL

Sensitivity: 3.26 pg/mL

Size: 5*96T, 96T, 15*96T

***This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before use.**

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.
CV(%) = SD/mean x 100

Intra/Inter-Assay Precision	Sample	n	Mean	Standard Deviation	CV%
Intra-assay	1	20	70.3	3.8	5.4
Intra-assay	2	20	195	9.4	4.8
Intra-assay	3	20	415	8.7	2.1
Inter-assay	1	20	70.8	5.5	7.7
Inter-assay	2	20	197	15.3	7.8
Inter-assay	3	20	414	26.4	6.4

Intended Use

This sandwich kit is for the accurate quantitative detection of Human Programmed cell death 1 ligand 1 (also known as PD-L1) in serum, plasma, cell culture supernatant. The other biological fluids need to be validated by the end user due to the complexity of natural targets and unpredictable interference.

Assay Principle

This is an Enzyme-Linked Immunosorbent Assay (ELISA) kit designed to detect Human PD-L1. The assay plate has been pre-coated with mouse anti-Human PD-L1 monoclonal antibody. When the sample containing PD-L1 is added to the plate, it binds to the antibodies coated on the wells. Then, a horseradish peroxidase conjugated mouse anti-Human PD-L1 Antibody is added to the wells and binds to PD-L1 in the sample. After washing the wells, substrate solutions are added, and the color intensity is directly proportional to the amount of Human PD-L1 present. The reaction is stopped by adding an acidic stop solution, and the absorbance is measured at 450 nm.

Reagents Provided

Components	Specifications	Storage condition
Pre-coated ELISA Plate	12 × 8 well strips × 1	Return unused wells to the foil pouch containing the desiccant pack and store for up to 1 month at 2-8℃ .
Standard Solution	0.2mL × 1 vail	Store at -20℃ in a manual defrost freezer until expiration date. Avoid repeated freeze-thaw cycles. Reconstituted working solution should be freshly prepared and discard after use.
Dilution Buffer	20mL × 1 vail	Unopened reagents can store at 2-8℃ until expiration date.Store opened reagents at 2-8℃ and use within one month.
Stop Solution	6mL × 1 vail	
Substrate Solution A	12mL × 1 vail	
Substrate Solution B	12mL × 1 vail	
Wash Buffer Concentrate (25x)	20mL × 1 vail	
Detection Antibody Concentrate	0.6mL × 1 vail	
User manual	1	
Plate Sealers	2pcs	
Zipper bag	1pcs	

Material Required But Not Supplied

- Absorbent paper
- Precision pipettes and disposable pipette tips
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 ± 10nm wavelength filter

Precautions

- This instruction must be strictly followed in the experiment.
- Store kit at 2 - 8°C. Do not use reagents after expiration date.
- All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.
- Substrate Solution B is sensitive to light, don't expose Substrate Solution B to light.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material.
- The kit should not be used beyond the expiration date.

Specimen Collection

Serum Allow serum to clot for 30 minutes at room temperature. Then centrifuge at 1,000 × g for 15 minutes. Collect the supernatant without sediment and assay immediately.
Plasma Collect plasma using EDTA or heparin as an anticoagulant. After mix 10-20 minutes, centrifuge samples for 15 minutes at 1,000 × g. Collect the supernatant without sediment and assay immediately.
Cell culture supernatant Remove particulates by centrifugation at 500 × g for 5 minutes. Collect the supernatant without sediment and assay immediately.

Note

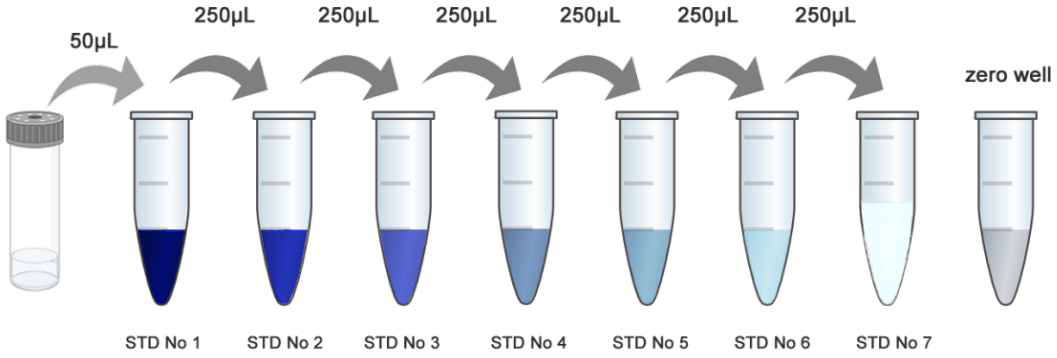
- Aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

- Samples should be brought to room temperature before starting the assay.
- Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

Reagent Preparation

- All reagents should be brought to room temperature (22 - 28°C) equilibration (at least 30 minutes) before use.
- **Standard Solution** Reconstitute 50µL of the standard solution (4 ng/mL) with 450µL of Dilution Buffer to generate a stock standard solution of 0.4ng/mL (Standard No.7). Allow the standard to sit for 15 minutes with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (0.4ng/mL) 1:2 with Dilution Buffer to produce 0.2ng/mL, 0.1ng/mL, 0.05ng/mL, 0.025ng/mL, 0.0125ng/mL and 0.00625ng/mL solutions. Dilution Buffer serves as the zero standard (0 pg/µL). Any remaining standard solution should be frozen at -20°C and used for up to one month. Dilution of standard solutions suggested are as follows:

Concentration	Standard No.	Dilution Steps
0.4ng/mL	Standard No.1	50µL Original Standard + 450µL Dilution Buffer
0.2ng/mL	Standard No.2	250µL Standard No.7 + 250µL Dilution Buffer
0.1ng/mL	Standard No.3	250µL Standard No.6 + 250µL Dilution Buffer
0.05ng/mL	Standard No.4	250µL Standard No.5 + 250µL Dilution Buffer
0.025ng/mL	Standard No.5	250µL Standard No.4 + 250µL Dilution Buffer
0.0125ng/mL	Standard No.6	250µL Standard No.3 + 250µL Dilution Buffer
0.00625ng/mL	Standard No.7	250µL Standard No.2 + 250µL Dilution Buffer
0ng/mL	Standard No.0	250µL Dilution Buffer



- **Wash Buffer** Add 20 µL of Wash Buffer Concentrate to 480 µL of deionized or distilled water to prepare 500 µL of 1× Wash Buffer. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.
- **Solution Substrate** Solution A and B should be mixed together in equal volumes within 10 minutes of use. Protect from light. Each well requires 200 µL of the resultant mixture.
- **Detection Antibody Solution** Add 0.6 mL of Detection Antibody Concentrate to 11.4 mL Dilution Buffer to prepare 12 mL Detection Antibody Solution.

Assay Procedure

1. Prepare all reagents, standard solutions, samples and Detection Antibody Solutions as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. It is recommended that all standards and samples be assayed in duplicate. Determine the number of strips needed for the assay and insert them into the frames provided. Store unused strips at 2-8°C.
2. Add 100 µL standard or sample per well. Cover the plate with a sealer and incubate 2 hours at room temperature.
3. Discard the contents of the plate. Add 300 µL of 1× Wash Buffer to each well and soak for 1 minute. Then aspirate or decant the liquid and pat the wells dry with clean paper towels. Repeat the process twice for a total of three washes. Ensure complete removal of liquid at each step. Note: Improper washes may lead to falsely elevated signals and poor reproducibility.
4. Add 100 µL of the Detection Antibody Solution to each well. Seal the plate and incubate 1 hour at room temperature.
5. Repeat the aspiration/wash as in Step 3.
6. Add 200 µL of Substrate Solution (Pre-mixed in reagent preparation step) to each well. Incubate for 20 minutes at room temperature. Protect from light.
7. Add 50 µL Stop Solution to each well to stop the reaction. The solution color in the wells should change from blue to yellow. Note: If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Summary

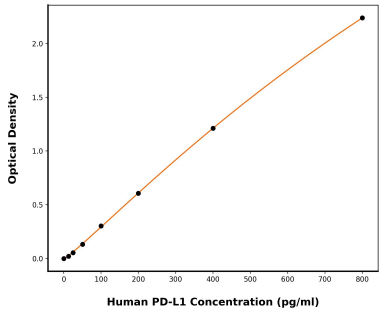
1. Prepare all reagents, standard solutions and samples.
2. Add standard or samples into each well. Incubate for 2 hours at room temperature.
3. Wash the plate 3 times.
4. Add Detection Antibody Solution into each well. Incubate for 1 hour at room temperature.
5. Wash the plate 3 times.
6. Add Substrate Solution. Incubate for 20 minutes at room temperature. Protect from light.
7. Add Stop Solution and color develops.
8. Read the OD value within 10 minutes.

Calculation of Result

1. If samples generate values higher than the highest standard, dilute the samples and repeat the assay, then the concentration read from the standard curve must be multiplied by the dilution factor.
2. Calculate the mean absorbance for each standard and sample, and subtract average zero standard optical density.
3. The data been calculated by 4-parameter logistics curve-fitting algorithm.

Typical Data

This standard curve is for demonstration purpose only. A standard curve should be generated with each assay.



Concentration	OD Value	Average	Corrected
800 pg/ml	2.334	2.323	2.239
	2.312		
400 pg/ml	1.287	1.295	1.211
	1.304		
200 pg/ml	0.662	0.691	0.607
	0.721		
100 pg/ml	0.388	0.386	0.302
	0.384		
50 pg/ml	0.218	0.217	0.133
	0.216		
25 pg/ml	0.134	0.138	0.054
	0.143		
12.5 pg/ml	0.099	0.105	0.021
	0.111		
0 pg/ml	0.087	0.084	0
	0.082		

Troubleshooting

Possible Case	Solution
High Background	
• Insufficient washes	• See washing procedure on the user manual • Increase number of washes
Weak Signal	
• Improper washing • Incorrect incubation temperature • Reagent are contaminated • Pipette are not clean	• Increasing duration of soaking steps • ncubate at room temperature • Use new one • Pipette should be clean
No Signal	
• Reagents added in incorrect order, or incorrectly prepared • Standard curve looks fine but there is no signal in the sample wells • Standard wells no signal but there is a signal in the sample wells	• Review manual and repeat assay • Check if the samples were handled and stored correctly. • Check that standard was handled according to manual.
Poor Standard Curve	
• Plate not developed long enough • Incorrect procedure • Imprecise / inaccurate pipetting	• Increase Substrate Solution incubation time • Review manual and repeat the assay to establish the standard curve. • Check and calibrate the pipettes

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