Human CD274 / PD-L1 ELISA Kit

Enzyme Immunoassay for the quantification of Human CD274 / PD-L1 in Human Serum, plasma (heparin, EDTA) and cell culture supernatants.

Catalog number: ARG81929

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

This gene encodes an immune inhibitory receptor ligand that is expressed by hematopoietic and non-hematopoietic cells, such as T cells and B cells and various types of tumor cells. The encoded protein is a type I transmembrane protein that has immunoglobulin V-like and C-like domains. Interaction of this ligand with its receptor inhibits T-cell activation and cytokine production. During infection or inflammation of normal tissue, this interaction is important for preventing autoimmunity by maintaining homeostasis of the immune response. In tumor microenvironments, this interaction provides an immune escape for tumor cells through cytotoxic T-cell inactivation. Expression of this gene in tumor cells is considered to be prognostic in many types of human malignancies, including colon cancer and renal cell carcinoma. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2015]
Involved in the costimulatory signal, essential for T-cell proliferation and production of IL10 and IFNG, in an IL2-dependent and a PDCD1-independent manner. Interaction with PDCD1 inhibits T-cell proliferation and cytokine production. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CD274 / PD-L1 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CD274 / PD-L1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for CD274 / PD-L1 is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added

to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of CD274 / PD-L1 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of CD274 / PD-L1 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C or at-20°C for long-term storage. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 X 10 ng/vial	4°C
Standard/Sample diluent	30 ml (Ready to use)	4°C
Antibody conjugate concentrate (100X)	1 vial (100 µl)	4°C
Antibody diluent buffer	12 ml (Ready to use)	4°C
HRP-Streptavidin concentrate (100X)	1 vial (100 µl)	4°C
HRP-Streptavidin diluent buffer	12 ml (Ready to use)	4°C
25X Wash Buffer	20 ml	4°C
TMB substrate	10 ml (Ready to use)	4°C (Protect from light)
STOP solution	10 ml (Ready to use)	4°C
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. The kit can also be stored at-20°C for long-term storage.
- To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
- The TMB Color developing agent should be colorless and transparent before using.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- If crystals are observed in the 25X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the 1X HRP-Streptavidin Solution and TMB substrate be pre-warmed in 37°C for few minutes before use.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

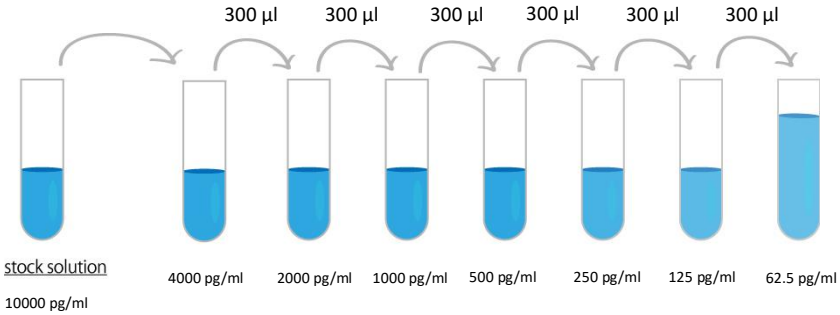
REAGENT PREPARATION

- 1X Wash Buffer: Dilute 25 X wash buffer with deionized water to yield 1X wash buffer. The pH value of dissolved 1X wash buffer should between pH7.2 to pH7.6. The diluted 1X wash buffer is stable for a week at 2°C to 8°C.
- 1X Antibody conjugate: It is recommended to prepare this reagent immediately prior to use and use it within 2 hours after preparation. Dilute 100X antibody conjugate concentrate into Antibody diluent buffer to yield 1X detection antibody solution. (e.g. 10 µl of 100X antibody conjugate concentrate + 990 µl of Antibody diluent buffer)
- 1X HRP-Streptavidin Solution: It is recommended to prepare this reagent immediately prior to use and use it within 1 hours after preparation. Dilute 100X HRP-Streptavidin concentrate solution into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer. (e.g. 10 µl of 100X HRP-Streptavidin concentrate solution + 990 µl of HRP-Streptavidin diluent buffer)
- Sample: If the initial assay found samples contain CD274 / PD-L1 higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluent buffer before assay.

(It is recommended to do pre-test to determine the suitable dilution factor).

- **Standards**: Standard solution should be prepared within 2 hours prior to the experiment. Reconstitute the standard with **1 ml** of **Standard/Sample diluent** to yield a stock concentration of **10000 pg/ml**. Allow the stock standard to sit for at least 10 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml.

  Note: The reconstituted standard solutions are best used within 2 hours. The stock standard solution should be stored at 4°C for up to 12 hours, or aliquot & store at-20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.



Dilute CD274 / PD-L1 standard as according to the table below:

Standard	CD274 / PD-L1 Conc. (pg/ml)	µl of Standard/Sample diluent	µl of standard
S7	4000 pg/ml	600	400 (10000 pg/ml Stock)
S6	2000 pg/ml	300	300 (S7)
S5	1000 pg/ml	300	300 (S6)
S4	500 pg/ml	300	300 (S5)
S3	250 pg/ml	300	300 (S4)
S2	125 pg/ml	300	300 (S3)
S1	62.5 pg/ml	300	300 (S2)
S0	0	300	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) or 37°C before use. The 1X HRP-Streptavidin Solution and TMB substrate should be prewarm at 37°C few minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard CD274 / PD-L1 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of CD274 / PD-L1 amount in samples. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards, samples and zero control** (S0, Standard/Sample diluent) into wells. Cover the plate and incubate for **90 minutes at 37°C.**
3. **Aspirate each well**. Complete removal of liquid by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time. Wash step is not necessary in this step.

4. Add **100 µl** of **1X Antibody conjugate** into each well, gently tap the plate to mix well. Cover wells and **incubate for 60 minutes at 37°C**.
5. Aspirate each well and wash, repeating the process two times for a total **three washes**. Wash by filling each well with **1X Wash Buffer** (or 0.01M PBS or TBS) **(300 µl)** using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.
6. Add **100 µl** of **1X HRP-Streptavidin solution** to each well, gently tap the plate to mix well. Cover wells and **incubate for 30 minutes at 37°C**.
7. Aspirate each well and wash, repeating the process four times for a total **five washes**. Wash by filling each well with **1X Wash Buffer** (or 0.01M PBS or TBS) **(300 µl)** using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.
8. Add **90 µl** of **TMB substrate** to each well. Incubate for **15-30 minutes at 37°C** in dark. (Note: The incubation time is for reference only, the optimal incubation time should be determined by end user. And the shades of blue color can be seen in the wells with the four most concentrated CD274 / PD-L1 standard solutions; the other wells show no obvious color).

9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
10. Read the OD with a microplate reader at 450 nm immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

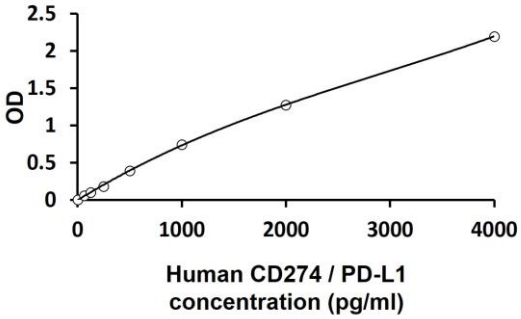
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human CD274 / PD-L1 ranged from 62.5 pg/ml- 4000 pg/ml. The mean MDD was 31.25 pg/ml.

Specificity

This assay recognizes natural and recombinant Human CD274 / PD-L1. No significant cross-reactivity or interference with the factors below was observed: There is no detectable cross-reactivity with other relevant proteins.

Intra-assay and Inter-assay precision

The CV values of intra-assay was 4.7% and inter-assay was 6%.