

For the quantitative determination of human PD-1 concentrations in cell culture supernates, serum, and plasma.

INTRODUCTION

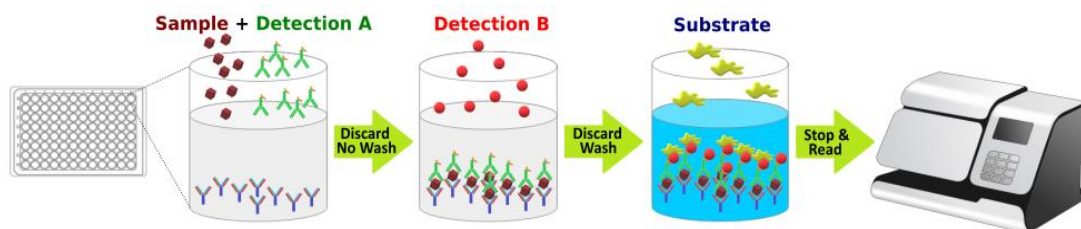
Programmed Cell Death Protein 1 (PD-1), a member of the CD28 family, is a type 1 transmembrane immunoglobulin protein consisting of 268 amino acids, encoded by a gene located on the long arm of chromosome 2. It comprises three domains: an extracellular domain (ectodomain) with 150 amino acids, a transmembrane region, and a cytoplasmic domain. PD-1 is primarily expressed on activated T cells, B cells, natural killer cells, dendritic cells, and macrophages, and functions as an immune checkpoint receptor that negatively regulates T cell activation and effector function. The interaction between PD-1 and its ligands, PD-L1 and PD-L2, plays a crucial role in anti-tumor immunity and cancer immunotherapy.

The Tribioscience Fast Human PD-1 ELISA is designed to quantitatively detect Human PD-1 levels in different tissues including skin, muscle, neural, serum, and other biological samples. The main feature is that **the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods.** It makes the assay simple, easy, accurate and fast. The measurement can be finished in 3 hours, not needing 4-5 hours (Fig. 1). The detection range is from 15 to 1000 pg/mL. The levels of human PD-1 samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human PD-1 protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (see Fig. 1). A monoclonal antibody specific for human PD-1 is pre-coated onto a microplate. Standards or samples and a biotin-conjugated detection antibody are pipetted into the wells and concurrently incubated to form a sandwich complex in one step. Simply aspirate each well without washing and directly add Streptavidin-HRP into the complex. Following a wash, an **ultra-sensitive TMB substrate solution** is added to the wells for color development. The color intensity is proportional to the amount of PD-1 bound in the initial step. The intensity of the color is measured by plate reading at 450 nm.

Fig. 1: Assay Principle:



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human PD-1 Microplate	TBS32116A	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human PD-1.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human PD-1 Standard	TBS32116B	30 µL of Recombinant human PD-1 protein (50 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS32116C	220 µL of Biotin-Human PD-1 antibody (10x).	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS32116D	300 µL of Streptavidin-HRP (50x).	
Assay Diluent	TBS32116E	15 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	15 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*)

Detection A working solution preparation: Add 210 µL of **Detection A Stock** (Biotin-human PD-1 antibody) to 1890 µL Assay Diluent to prepare Detection A working solution. Add 20 µL to each well.

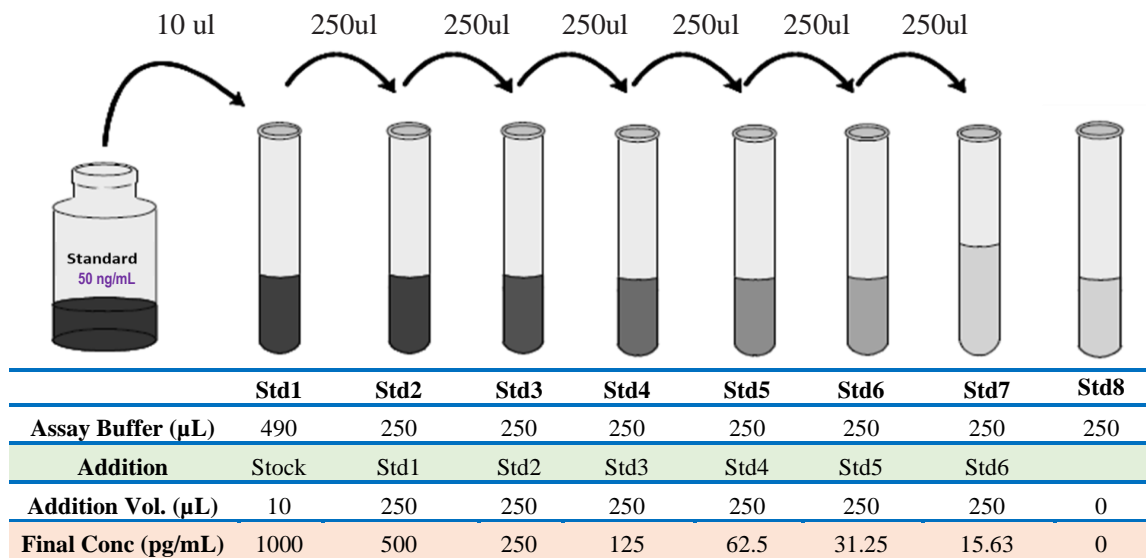
Detection B working solution preparation: Add 240 µL of **Detection B** streptavidin-HRP to 12 mL Assay Diluent to prepare Detection B working solution. Add 100 µL to each well.

Human PD-1 Standard Preparation:

Label test tubes as #1 through #8. Pipet 490 µL of 1x Assay Diluent into tube #1, and 250 µL into tubes #2 to #7 as diagram below (Fig.2).

1. Add 10 µL of the Human PD-1 Standard stock solution (50 ng/mL) by dilution of 50X to tube #1 (1000 pg/mL), and mix.
2. Make 2x serial dilutions of the standard using the 1000 pg/mL standard solution (tube #1) from tube #2 through #7 with sequential transfer of 250 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tubes 1 through 7 will be 1000, 500, 250, 125, 62.5, 31.25, and 15.63 pg/mL. Tube # 8 is Standard 0.

Fig.2 Diagram for Human PD-1 standard preparation



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B** to each well. Incubate at **RT for 1 hour**.
5. Aspirate each well, and wash for 3 times by filling each well with 200 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 100 μ L of **TMB Substrate** to each well. Incubate **at RT for 10-20 min** (*Protect from light*). The color becomes blue.
7. Add 50 μ L of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human PD-1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve ($R^2=0.9995$) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

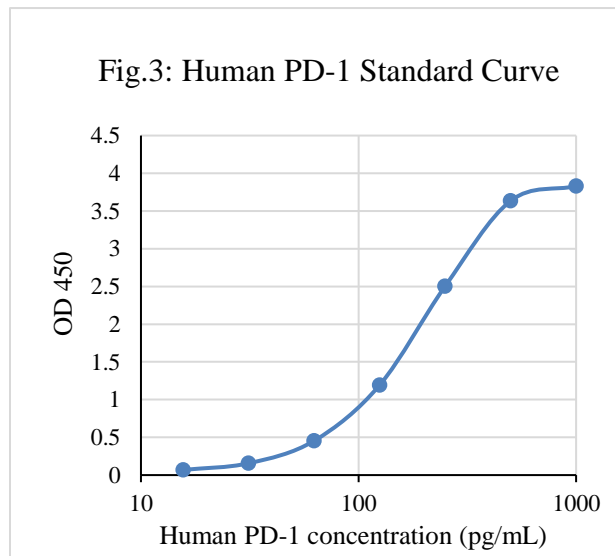
The minimum detectable dose (MDD) of human PD-1 is typically 10 pg/mL.
The Intra-assay CV is 3.79% the Inter-assay CV is <10%.

SPECIFICITY

This assay recognizes natural and recombinant human PD-1.

RELATIVE PRODUCTS

- Human IL-1 β ELISA (TBS3219)
- Human IL-2 ELISA (TBS3220)
- Human IL-4 ELISA (TBS3221)
- Human IL-6 ELISA (TBS3223)
- Human IL-7 ELISA (TBS3224)
- Human IL-8 ELISA (TBS3225)
- Human IL-10 ELISA (TBS3226)
- Human IL-13 ELISA (TBS3227)
- Human IL-17 ELISA (TBS3228)
- Human IL-22 ELISA (TBS3229)
- Human IFN-gamma ELISA (TBS3230)
- Human TGF- β 1 ELISA (TBS3232)
- Human GM-CSF ELISA (TBS3233)
- Human MIP-1 α ELISA (TBS3234)



For research use only.