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Mouse PD-1 ELISA Kit

Catalog number: NR-E10628 (96 wells)

The kit is designed to quantitatively detect the levels of Mouse PD-1 in ***cell culture supernatants.***

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 20 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

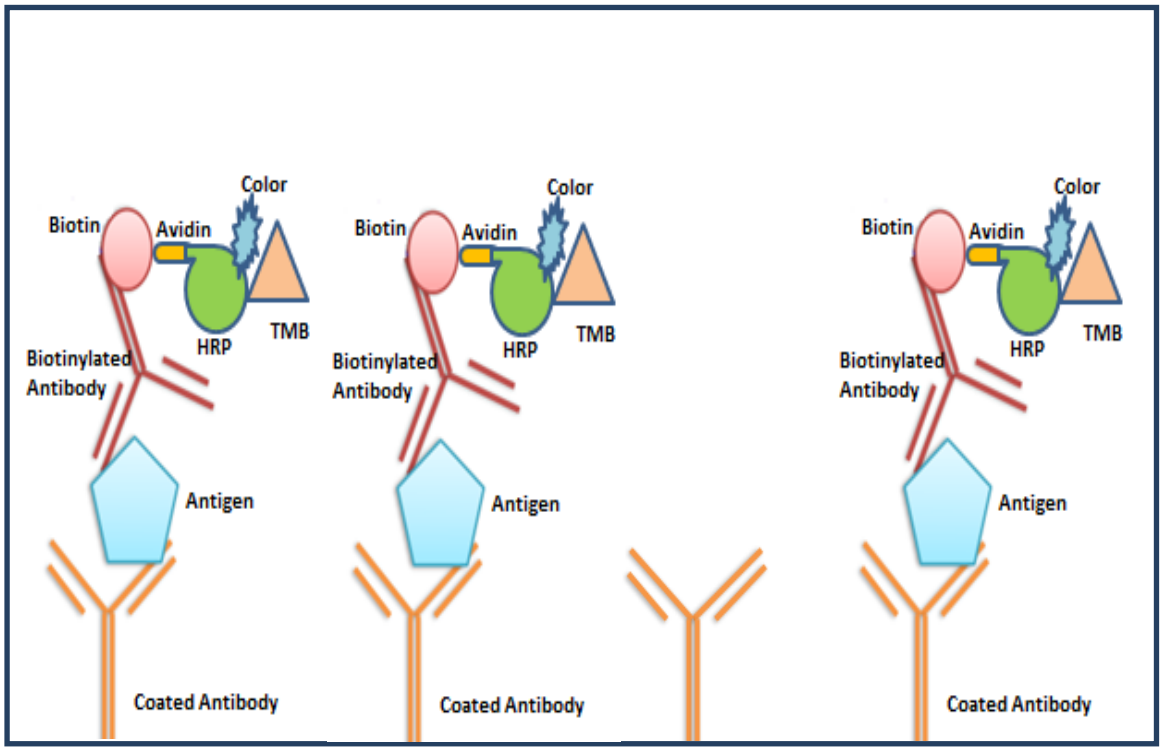
Intended use

The kit is used to quantify the Mouse PD-1 in cell culture supernatants.

Standard range	125-8000 pg/ml
Sensitivity	100 pg/ml
Assay time	4 hours
Validity	Six months
Store at	2-8 °C

Assay principle

This Mouse PD-1 ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Mouse PD-1 specific antibody has been precoated onto 96-well plate. The test samples and the biotinylated Mouse PD-1 specific detection antibody are added to the wells subsequently and then followed by washing the plate. Streptavidin-HRP is added and unbound conjugates are washed away with Wash Buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic Stop Solution. The density of yellow is proportional to the Mouse PD-1 amount of sample captured in plate.



Materials supplied

1.Mouse PD-1 standard:	2 ng/vial ×2.
2. 96-well plate coated with anti-Mouse PD-1 Ab:	1.
3. Sample diluent buffer:	12 ml× 2.
4. Biotinylated Mouse PD-1 Ab:	1 vial, dilution 1:20.
5. Streptavidin-HRP:	1 vial.
6. Antibody diluent buffer:	12 ml.
7. Streptavidin-HRP diluent buffer:	12 ml.
8. TMB developing agent:	12 ml.
9. Stop Solution:	6 ml.
10. 20 × Wash Buffer:	25 ml.
11. Plate sealer	1.
12. Package insert	1.

Materials required but not supplied

- 37°C incubator.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.

Sample Preparation and storage

Cell culture supernatant: Remove particulates by centrifugation at 3000 x g for 10 minutes, analyze immediately or aliquot and store at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. **The user should determine the optimal dilution factor**.

Reagent Preparation.

Standard

- Mouse PD-1: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (2 ng /vial) are included in each kit. Use one tube for each experiment.
- 8000 pg/ml→125 pg/ml of Mouse PD-1 standard solutions:
- Add 1 ml of sample diluent into one standard tube with 2 ng Mouse PD-1. Keep the tube at room temperature for 10 minutes and mix thoroughly. This is 20,000 pg/ml standard solution.
- Label 7 Eppendorf tubes with 8000 pg/ml, 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, and 125 pg/ml, respectively. Add 400 µl of 20,000 pg/ml standard solution and 600 µl of diluent buffer into 8000 pg/ml tube, mix well, This is 8,000 pg/ml standard solution, then make 2-fold serial dilution from 8000 pg/ml to 125 pg/ml in seven 1.5 ml tubes.
- Make sure each tube has ≥ 300 µl of standard.

Note: The standard solutions are best used within 2 hours.

Biotinylated Mouse PD-1 antibody working solution

- The solution should be prepared no more than 2 hours prior to the experiment.
- The total volume should be 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume.
- Spin down before opening the vial. Biotinylated anti-Mouse PD-1 detection antibody should be diluted in 1:20 with Antibody diluent buffer. Allow the diluted Detection Antibody to sit at least 1-2 hours before use.

Streptavidin-HRP working solution

- The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume).
- Spin down before opening the vial. Streptavidin-HRP should be diluted in 1:100 with Streptavidin-HRP diluent buffer and mixed thoroughly.

Wash Buffer

- If crystals have formed in the 20 × wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20 ×) to a total volume of 500ml with distilled water.

Assay procedures

Bring all reagents to room temperature before use. Mouse PD-1 Standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of Mouse PD-1 concentration in samples.

1. Add 100 µl of sample or standards per well. Add 100 µl of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate 2 hours at room temperature. Note: We recommend that each Mouse PD-1 standard solution and each sample is measured in duplicate.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µl of biotinylated Mouse PD-1 antibody working solution to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 for five times.
7. Add 100 µl of TMB developing agent to each well. Cover and incubate for 20-40 minutes at room temperature (Protect from light. Do not over-develop).
8. Add 50 µl Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Result calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Mouse PD-1 concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Background

Programmed Death-1 (PD-1), also known as Programmed Cell Death-1, or CD279, is a type I transmembrane protein belonging to the CD28/CTLA-4 family. The human PD1 is synthesized as a 288 amino acid (aa) protein containing a putative 20 aa signal peptide, a 148 aa extracellular region with one immunoglobulin-like V-type domain, a 24 aa transmembrane domain and a 95 aa cytoplasmic region which consists of two tyrosine residues that form the immuno receptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM) that play a role in mediating PD-1 signaling. PD-1 is expressed on activated T cells, B cells, myeloid cells and on a subset of thymocytes. Human PD-1 shares approximately 60% aa sequence identity with mouse counterpart.

PD-L1/B7H1 and PD-L2/B7DC, members of the B7 family, are the ligands for PD-1. PD-L1 protein is upregulated on macrophages and dendritic cells (DC) in response to LPS and GM-CSF treatment, and on T cells and B cells when TCR and B cell receptor is activated. PD-L1 is expressed on almost all murine tumor cell lines, such as PA1 myeloma, P815 mastocytoma, and B16 melanoma upon treatment with IFN-γ. PD-L2 is expressed mainly by DCs and a few tumor lines. It is reported that ligation of PD-1 inhibits TCR-mediated T cell proliferation and production of IL-1, IL-4, IL-10,and IFNγ, as well as BCR mediated signaling pathways. In addition, PD-1 deficient mice have shown a defect in peripheral tolerance and spontaneously develop autoimmune diseases, suggesting that PD-1 might play a role in the regulation of peripheral tolerance and in preventing autoimmune diseases.

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