# TECHNICAL MANUAL

# Mouse PD-1 ELISA Kit

- **SKU CODE:** SBRS7788
- **SIZE:** 96 Wells
- **DETECTION PRINCIPLE:** Sandwich ELISA
- **RUO:** Research-Use-Only

Revised: 11/26

# Mouse PD-1 ELISA Kit

*Please read entire manual carefully before starting experiment.*

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# 1. Key Features

**Detection Method:**

Sandwich

**Sample Type:**

Serum, plasma, and cell culture supernatants.

**Reactivity:**

Mouse PD-1

**Sensitivity:**

0.11 ng/mL

# 2. Storage & Expiry

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see the Materials Provided table. Date of expiration is on the ELISA Box label.

# 3. Principle of the Assay

This assay employs an antibody specific for mouse PD-1 coated on a 96-well plate. Standards and samples are pipetted into the wells and PD-1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse PD-1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of PD-1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

# 4. Materials Provided

| Component | Quantity | Description | Storage After Preparation |
|---|---|---|---|
| Mouse PD-1 Microplate* | 1 plate | 96 wells (12 x 8) coated with anti-Mouse PD-1 | 1 month at 4°C |
| Mouse PD-1 Standard Protein | 2 vials | Each vial is enough to run each standard in duplicate | 1 week at -80°C |
| Mouse PD-1 Detection Antibody | 2 vials | Each vial is enough to assay half the microplate | 5 days at 4°C |
| Wash Buffer (20X) | 25 mL | 20X concentrated solution | 1 month at 4°C |
| HRP-Streptavidin | 200 µL | 100X concentrated HRP-conjugated streptavidin | Do not store and reuse |
| TMB One-Step Substrate Reagent | 12 mL | 3,3,5,5'-tetramethylbenzidine in buffer solution | N/A |
| Stop Solution | 8 mL | 0.2 M sulfuric acid | N/A |
| Assay Diluent B (5X) | 15 mL | 5X concentrated diluent buffer | 1 month at 4°C |
| 96-Well Plate Sealing Film** | 2 films | Microplate adhesive sealing film | N/A |

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

**Optional use for overnight incubations.

# 5. Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µL to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- 100 mL and 1 L graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard or sample dilutions
- Plastic wrap
-

# 6. Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Assay Diluent B (5X) should be diluted 5-fold with deionized or distilled water before use.
3. **Sample dilution:** Assay Diluent B (1X) should be used for dilution of serum, plasma and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2-fold.

   ***Note:*** *Levels of PD-1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.*

**4. Preparation of standard:** Briefly spin a vial of standard protein. Add 640 µL Assay Diluent B (1X) (should be diluted 5-fold with deionized or distilled water before use) into the Standard Protein vial to prepare a 30 ng/mL standard solution. Dissolve the powder thoroughly by a gentle mix. Pipette 300 µL Assay Diluent B (1X) into each tube. Use the 30 ng/mL standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent B (1X) serves as the zero standard (0 ng/mL).

| | Std 1 | Std 2 | Std 3 | Std 4 | Std 5 | Std 6 | Std 7 | Zero Std |
|---|---|---|---|---|---|---|---|---|
| **Diluent volume** | Standard + 640 µL | 300 µL | 300 µL | 300 µL | 300 µL | 300 µL | 300 µL | 300 µL |
| **Conc.** | 30 ng/mL | 12 ng/mL | 4.8 ng/mL | 1.92 ng/mL | 0.768 ng/mL | 0.307 ng/mL | 0.123 ng/mL | 0 ng/mL |

5. If the Wash Buffer (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer (20X) into deionized or distilled water to yield 400 mL of Wash Buffer (1X).

6. Briefly spin a vial of detection antibody. Note that the volume of this component is very low and may not be visible by eye. Add 100 µL of Assay Diluent B (1X) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with Assay Diluent B (1X) and used in step 5 of the assay procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 100-fold with Assay Diluent B (1X).

***For example:*** *Briefly spin the HRP-Streptavidin vial and pipette up and down to mix gently. Add 100 µL of HRP-Streptavidin concentrate into a tube with 10 mL Assay Diluent B (1X) to prepare a 100-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.*

# 7. Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µL of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover wells with plastic wrap and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with Wash Buffer (1X). Wash by filling each well with Wash Buffer (1X) (300 µL) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential. After the last wash, remove any remaining Wash Buffer (1X) by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of 1X prepared detection antibody (Reagent Preparation step 6) to each well. Cover wells with plastic wrap and incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Cover wells with plastic wrap and incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µL of TMB One-Step Substrate Reagent to each well. Cover wells with plastic wrap and incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

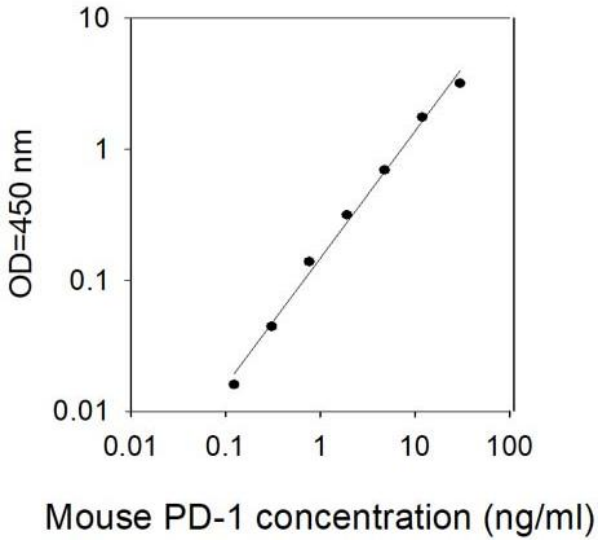
# 8. Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using SigmaPlot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

# 9. Typical Data

**Standard Curve**

These standard curves are for demonstration only. A standard curve must be run with each assay.




**Precision**

- Intra-Assay CV%: <10%
- Inter-Assay CV%: <12%

**Recovery**

Recovery was determined by spiking various levels of Mouse PD-1 into the sample types listed below. Mean recoveries are as follows:

| Sample Type | Average % Recovery | Range (%) |
|---|---|---|
| Serum | 74.19 | 71-78 |
| Plasma | 75.74 | 70-83 |
| Cell culture media | 108.8 | 103-120 |

**Linearity**

| Dilution | | Serum | Plasma | Cell Culture Media |
|---|---|---|---|---|
| **1:2** | Average % of Expected | 104.5 | 123.4 | 108.7 |
| | Range (%) | 101-108 | 123-125 | 106-112 |
| **1:4** | Average % of Expected | 131.3 | 131.2 | 132.1 |
| | Range (%) | 127-136 | 128-134 | 130-134 |

# 10. Troubleshooting Guide

| Problem | Cause | Solution |
|---|---|---|
| **Poor standard curve** | Inaccurate pipetting | Check pipettes |
| | Improper standard dilution | Briefly centrifuge the standard protein and dissolve the powder thoroughly by gently mixing |
| **Low signal** | Improper preparation of standard and/or biotinylated antibody | Briefly spin down vials before opening. Dissolve the powder thoroughly. |
| | Too brief incubation times | Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background). |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| **Large CV** | Inaccurate pipetting | Check pipettes |
| | Air bubbles in wells | Remove bubbles in wells |
| **High background** | Plate is insufficiently washed | Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. |
| | Contaminated wash buffer | Make fresh wash buffer |
| **Low sensitivity** | Improper storage of the ELISA kit | Store your standard at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light. |
| | Stop solution | Add stop solution to each well before reading plate |

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If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.