Version: Datasheet-KE10155-A.0

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# Mouse CD2 Sandwich ELISA Kit Datasheet

Please read it entirely before use

**Catalogue Number:** KE10155

**Size:** 96T

**Sensitivity:** 2.2 pg/mL

**Range:** 23.4-1500 pg/mL

**Usage:** For the quantitative detection of mouse CD2 concentrations in serum, plasma and tissue lysate.

**This product is for research use only and not for use in human or animal therapeutic or diagnostic.**



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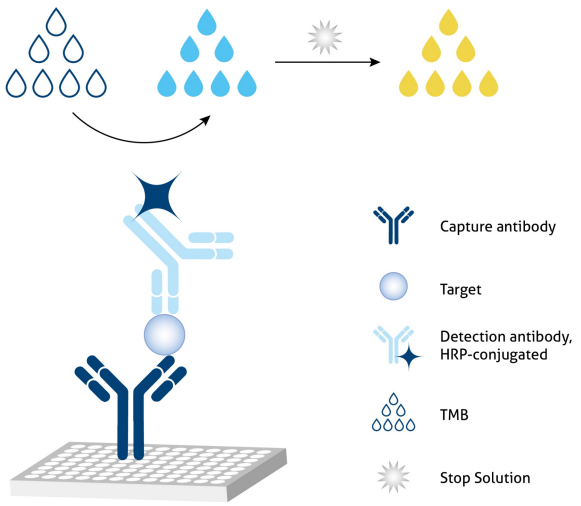
## 1. Background

CD2 is a transmembrane glycoprotein of the immunoglobulin superfamily expressed on the surface of T cells, NK cells, thymocytes, and dendritic cells. The CD2 antigen was identified as one of the earliest T cell markers and was shown to be important for thymocyte development. CD2 stimuli were mandatory for Treg cell survival with reduced Bim expression. Moreover, its down-regulation may attenuate anti-tumor T-cell responses with implications for checkpoint immunotherapies.

## 2. Principle




**Sandwich ELISA structure (Detection antibody labeled with HRP)**

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

## 3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.



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## 4. Kit Components and Storage

| | | |
|---|---|---|
| **Microplate** - antibody coated 96-well microplate (8 well × 12 strips) | 1 plate | **Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.** |
| **Protein standard** - 3000 pg/bottle; lyophilized | 2 bottles | |
| **Detection antibody, HRP-Conjugated (100×)** - 120 uL/vial* | 1 vial | |
| **Sample Diluent PT 4B1** - 30 mL/bottle | 2 bottles | |
| **Detection Diluent** - 30 mL/bottle | 1 bottle | |
| **Wash Buffer Concentrate (20×)** - 30 mL/bottle | 1 bottle | |
| **Extraction Reagent** - 30 mL/bottle | 1 bottle | |
| **Tetramethylbenzidine Substrate (TMB)** - 12 mL/bottle | 1 bottle | |
| **Stop Solution** - 12 mL/bottle | 1 bottle | |
| **Plate Cover Seals** | 4 pieces | |

* Centrifugation immediately before use

## 5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

## 6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.



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## 7. Reagent Preparation

**7.1 Wash Buffer (1X):** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

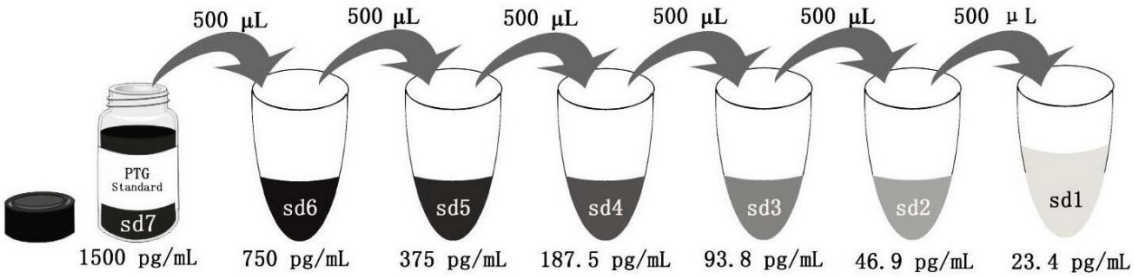
**7.2 Detection Antibody, HRP-conjugated(1X):** Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 uL 100X Detection Antibody, HRP-conjugated + 990 uL Detection Diluent (Centrifuge the 100X Detection Antibody solution, HRP-conjugated for a few seconds prior to use).

**7.3 Sample Dilution:** Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:4 or 1:8 is recommended for mouse serum and plasma; 1:128 or 1:256 is recommended for tissue lysate.

**7.4 Standard Serial Dilution:**

Add 2 mL Sample Diluent PT 4B1 in protein standard.




| Add # µL of Standard diluted in the previous step | — | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
|---|---|---|---|---|---|---|---|
| # µL of Sample Diluent PT 4B1 | **2000 µL** | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |



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## 8. Assay Procedure Summary

**Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.**

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.8 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.9 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.10 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

| Step | Reagent | Volume | Incubation | Wash | Notes |
|---|---|---|---|---|---|
| 1 | Standard and Samples | 100 uL | 120 min | 4 times | Cover Wells incubate at 37°C |
| 2 | Diluent Detection antibody, HRP-conjugated Solution | 100 uL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 3 | TMB Substrate | 100 uL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 4 | Stop Solution | 100 µL | 0 min | Do not wash | - |
| 5 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |



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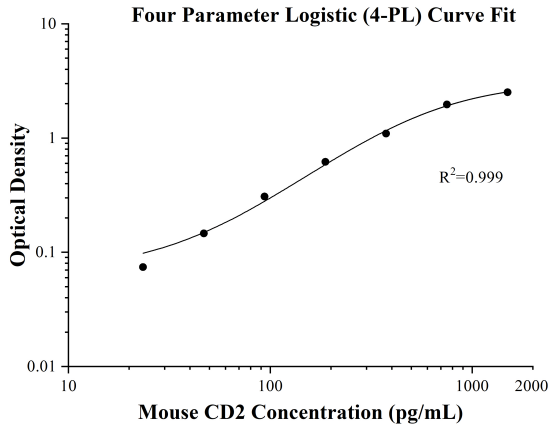
# 9. Validation Data

## 9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.




| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.0154<br>0.0119 | 0.014 | - |
| 23.4 | 0.0921<br>0.0828 | 0.087 | 0.074 |
| 46.9 | 0.1577<br>0.1625 | 0.16 | 0.146 |
| 93.8 | 0.3139<br>0.3303 | 0.322 | 0.308 |
| 187.5 | 0.6228<br>0.6447 | 0.634 | 0.62 |
| 375 | 1.061<br>1.1506 | 1.106 | 1.092 |
| 750 | 1.961<br>2.0033 | 1.982 | 1.969 |
| 1500 | 2.535<br>2.5239 | 2.529 | 2.516 |

## 9.2 Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

| Intra-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 8 | 733.2 | 41.7 | 5.7 |
| 2 | 8 | 183.5 | 8.7 | 4.8 |
| 3 | 8 | 88.9 | 4.3 | 4.9 |

| Inter-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 16 | 715.4 | 44.5 | 6.2 |
| 2 | 16 | 180.9 | 11.6 | 6.4 |
| 3 | 16 | 88.0 | 4.8 | 5.5 |

## 9.3 Recovery

The recovery of mouse CD2 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | | Average% of Expected | Range (%) |
|---|---|---|---|
| Mouse serum | 1:32 | 110 | 98-114 |
| Tissue lysate | 1:1,024 | 99 | 95-106 |



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## 9.4 Sample values

**Mouse serum** - mouse serum samples were evaluated for the presence of mouse CD2 in this assay.

| Sample Type | Mean (pg/mL) | Range (pg/mL) |
|---|---|---|
| Mouse serum (n=16) | 965.0 | 640.3-1,465.4 |

**Tissue lysate**

| | Mouse CD2 (**ng/mL**) | Total protein (**mg/mL**) |
|---|---|---|
| Mouse thymus tissue lysate | 119.7 | 1.3 |
| Mouse spleen tissue lysate | 67.0 | 2.9 |

## 9.5 Sensitivity

The minimum detectable dose of mouse CD2 is 2.2 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

## 9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The mouse serum was initially diluted 1:2. The tissue lysate was initially diluted 1:64.)

| | | Mouse serum | Tissue lysate |
|---|---|---|---|
| 1:2 | Average% of Expected | 100 | 100 |
| | Range (%) | - | - |
| 1:4 | Average% of Expected | 114 | 101 |
| | Range (%) | 107-119 | 99-103 |
| 1:8 | Average% of Expected | 124 | 107 |
| | Range (%) | 123-125 | 105-108 |
| 1:16 | Average% of Expected | 125 | 106 |
| | Range (%) | 121-128 | 105-107 |

## 9.7 Specificity

This assay recognizes natural and recombinant mouse CD2.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:

CD2



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## 10. References


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