DATA SHEET

Mouse Prostaglandin E2(PGE2) ELISA Kit

Catalog No.: 20361

96 T

For Research Use Only

Not for Use In Diagnostic Procedures

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDRUE

Intended use: The ELISA Kit is to be used for quantitative determination of MouseProstaglandin E2(PGE2) in vitro.

1. Principles of Method

The Mouse PGE2 ELISA Kit is an Vitro enzyme-linked immunosorbent assay for the quantitative measurement of Mouse PGE2 in serum, plasma, tissue,cell culture supernatants and urine. The Mouse PGE2 polyclonal antibodies are precoated onto 96-well plate. Standard and samples are pipetted into the wells and Mouse PGE2 present in a sample is bound to the wells by the immobilized antibody. The biotinylated detection antibodies are added to the wells and then followed by washing with PBS or TBS buffer. After washing away unbound biotinylated antibody, Avidin-Biotin -Peroxidase Complex is pipetted to the wells. The wells are washed again, a TMB substrate solution is added to the wells and the color changes after adding acidic stop solution. The indensity is proportional to the amount of Mouse PGE2 bound and measured at 450nm±10nm.

2. Reagents Supplied

① Pre-coated Microplate: 96 wells (12stripsx8 wells) coated with anti-Mouse PGE2.
② Standards: 96 tests(2 vials)
③ Detection Antibody Mouse PGE2：96 tests （1：100）
④ Avidin-Biotin-Peroxidase Complex(ABC): 96 tests（1：100）
⑤ Sample Diluent Buffer : 96 tests (2 vials)
⑥ Antibody Diluent Buffer: 96 tests (1 vials)
⑦ ABC Diluent Buffer: 96 tests (1 vials)
⑧ TMB color developing reagent A: 96 tests (1 vials)
⑨ TMB color developing reagent B: 96 tests (1 vials)
⑩ TMB stop solution: 96 tests (1 vials)
⑪ ELISA Special TBS Diluent: 96 tests (1 vials,1:25)

3. Materials Required But Not Provided

① Microtiter plate reader in standard size.
② Polypropylene tubes for diluting and aliquoting standard
③ Distilled or deionized water
④ Calibrated, adjustable precision pipettes, preferably with disposable plastic tips

4. Performance Characteristic

Normal Range: 15.6pg/ml - 1000pg/ml

Sensitivity: < 5pg/ml

Specificity: The ELISA Kit shows no cross reactivity with any of the cytokines.

5. Storage and stability

All the components in the kit can be stored up to 1 year at -20℃ and 4 weeks at 2-8℃. Please avoid repeated freeze-thaw cycles and do not mix reagents from different kits unless they have the same lot numbers.

6. Reagent Preparation

① Plate washing

Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.35 ml PBS or TBS buffer for 1~2 minutes ,then discard the rinse solution. Repeat this process t for several times.

② Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernate, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C

Serum: Allow the serum to clot in a serum separator tube (about 2 hours or 4℃) at room temperature. Centrifuge at approximately 1000 X g for 10 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

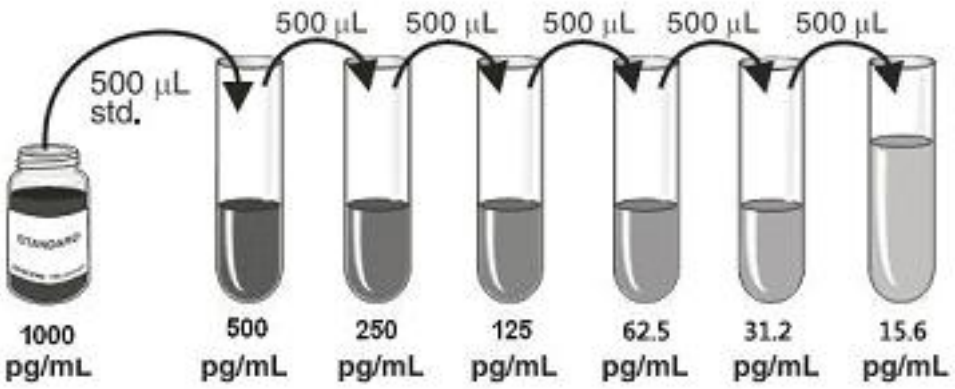
Plasma: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

Sample Dilution Guideline

User needs to estimate the concentration of the target protein in the sample and select proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

③ Reagent Preparation and Storage

A . Preparation of the standard： Standard solution should be prepared no more than 2 hours prior to the experiment. Add 1 ml sample diluent buffer into one tube, dissolve the standard thoroughly and make times dilution.



B. Preparation of biotinylated anti-Mouse PGE2 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

b. Biotinylated anti-Mouse PGE2 antibody should be diluted in 1:99 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

b. Avidin-Biotin-Peroxidase Complex (ABC) should be diluted in 1:99 with the ABC dilution buffer and mixed thoroughly.

D. Preparation of TMB working solution: transfer 9 volumes of TMB color developing reagent A in one volume of TMB color developing reagent B for 30 minutes in 37℃ before using to make TMB substrate, mixing thoroughly.

7. Assay Procedure

The user should decide sample dilution fold by crude estimation of Mouse PGE2 amount in samples.

① Aliquot 0.1ml per well of the grades Mouse PGE2 standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well . Add 0.1ml of each properly diluted sample of sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well.

② Seal the plate with the cover and incubate at 37°C for 90 min.

③ Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time, washing twice.

④ Add 0.1ml of biotinylated anti-Mouse PGE2 antibody working solution into each well and incubate the plate at 37°C for 60 min.

⑤ Wash the plate three times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min.

⑥ Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.

⑦ Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min.

⑧ Add 0.09 ml of prepared TMB color developing agent into each well and incubate plate at 37°C away from light, observe the color at all times, when shades of blue can be seen in the wells with the three-four most concentrated Mouse PGE2 standard solutions; the other wells show no obvious color, Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.

⑨ Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

⑩ 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 PGE2 concentration of the samples can be interpolated from the standard curve.

Summary

prepare reagents, samples and standards

⇩

Add prepared samples or standards and incubate the plate at 37℃ for 90min, wash plate twice with TBS

⇩

Add biotinylated antibodies and incubate the plate at 37℃ for 60min, wash plate 3 times with TBS or PBS

⇩

Add ABC working solution and incubate the plate at 37℃ for 30 min, wash plate five times with TBS or PBS

⇩

Add TMB color developing agent and incubate at 37℃

⇩

Add TMB stop solution

⇩

Read the O.D. absorbance at 450nm within 30 min

⇩

Calculate the Mouse PGE2 concentration in the samples