# Mouse Prostaglandin E2 (PG-E2)ELISA Kit

**Catalog No. CSB-E07966m**

(96T)

- This immunoassay kit allows for the in vitro quantitative determination of **mouse PG-E2** concentrations in **serum, plasma and tissue homogenates**.
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

www.cusabio.com

## PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with an goat-anti-rabbit antibody. Standards or samples are then added to the appropriate microtiter plate wells with a HRP-conjugated PG-E2 and antibody preparation specific for PG-E2 and incubated. Then substrate solutions are added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of PG-E2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

## DETECTION RANGE

0.4 pg/ml-80 pg/ml. The standard curve concentrations used for the ELISA's were 80 pg/ml, 20 pg/ml, 6.4 pg/ml, 1.6 pg/ml, 0.4 pg/ml。

## SPECIFICITY

This assay recognizes mouse PG-E2. No significant cross-reactivity or interference was observed.

## SENSITIVITY

The minimum detectable dose of mouse PG-E2 is typically less than 0.2 pg/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

## MATERIALS PROVIDED

| **Reagent** | **Quantity** |
|---|---|
| Assay plate | 1 |
| Standards (S0-S5) | 6 x 0.5 ml |
| HRP-conjugate | 1 x 6 ml |
| Antibody | 1 x 6 ml |
| Wash Buffer | 1 x 15 ml (20×concentrate) |
| Substrate A | 1 x 7 ml |
| Substrate B | 1 x 7 ml |
| Stop Solution | 1 x 7 ml |

| Standard | S 0 | S 1 | S 2 | S 3 | S 4 | S 5 |
|---|---|---|---|---|---|---|
| Concentration(pg/ml) | 0 | 0.4 | 1.6 | 6.4 | 20 | 80 |

## STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

## TECHNICAL HINTS

1. Bring all reagents and plate to room temperature for at least 30 minutes before use. Unused wells need store at 2-8℃and avoid sunlight.
2. **Wash Buffer** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 300 ml of Wash Buffer.

3. 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.
4. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Sealers can not be reused.
6. Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
7. Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

***Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.***

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

## SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20° C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.
- **Plasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

- **Tissue Homogenates** 100mg tissue was rinsed with 1X PBS, homogenized in 1 mL of 1X PBS and stored overnight at -20° C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. The supernate was assayed and removed immediately. Alternatively, aliquot and store samples at -20°C or -80℃. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

***Note: Grossly hemolyzed samples are not suitable for use in this assay.***

## ASSAY PROCEDURE

***Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.***

1. Set a Blank well without any solution. Add 50μl of Standard or Sample per well. Standards need test in duplicate.
2. Add 50μl of **HRP-conjugate** to each well (not to Blank well), then add 50μl **Antibody** to each well. Mix well and then incubate for 1 hour at 37°C.
3. Fill each well with **Wash Buffer** (about 200μl), stay for 10 seconds and Spinning. Repeat the process for a total of three washes. 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.

4. Add 50μl of **Substrate A** and **Substrate B** to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
5. Add 50μl of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
6. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

## CALCULATION OF RESULTS

***Using the professional soft "Curve Exert 1.3" to make a standard curve is recommended, which can be downloaded from our web.***

Average the duplicate readings for each standard, Blank, and sample and subtract the optical density of Blank. Create a standard curve by reducing the data 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 x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the

log of the PG-E2 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. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in Standard Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.