

Rat Prostaglandin E2 (PGE2) ELISA Kit

Catalog No. CSB-E07967r

(96T)

- This immunoassay kit allows for the in vitro quantitative determination of **rat PGE2** concentrations in **tissue homogenates**.
- **Expiration date** six months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

INTRODUCTION

One of the prostaglandins, a group of hormone-like substances that participate in a wide range of body functions such as the contraction and relaxation of smooth muscle, the dilation and constriction of blood vessels, control of blood pressure, and modulation of inflammation. Prostaglandin E2 (PGE-2) is released by blood vessel walls in response to infection or inflammation that acts on the brain to induce fever. The enzyme mPGES-1 is involved in the production of PGE2 and is an important "switch" for activating the fever response.

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 PGE2 and antibody preparation specific for PGE2 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 $450 \text{ nm} \pm 2$

nm. The concentration of PGE2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

DETECTION RANGE

0.4 pg/ml-100 pg/ml. The standard curve concentrations used for the ELISA's were 100 pg/ml, 25 pg/ml, 6.25 pg/ml, 1.55 pg/ml, 0.4 pg/ml.

SPECIFICITY

This assay recognizes recombinant and natural rat PGE2. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of rat PGE2 is typically less than 0.25 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	Storage
Unopened kit	Store at 2 - 8° C. Do not use beyond kit expiration date.		
Opened kit	Coated plate	1	May be stored for up to 1 month at 2 - 8° C. Try to keep it in a sealed aluminum foil bag, and avoid the damp
	Standards (S1-S5)	5 x 0.5 ml	
	Antibody	1 x 6 ml	May be stored for up to 1 month at 2 - 8° C.
	HRP-conjugate	1 x 6 ml	
	Wash Buffer	1 x 15 ml (20xconcentrate)	
	Substrate A	1 x 7 ml	
	Substrate B	1 x 7 ml	
	Stop Solution	1 x 7 ml	

Standard	S 1	S 2	S 3	S 4	S 5
Concentration (pg/ml)	0.4	1.55	6.25	25	100

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°C 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

- **Tissue Homogenates** The preparation of tissue

homogenates will vary depending upon tissue type. Heart and lung tissue from one rat was rinsed with 1X PBS to remove excess blood, homogenized in 20 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. The supernate was assayed and removed immediately. Alternatively, aliquot and store samples at -20° C. 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 y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the PGE2 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.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.

- 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.

PRECISION

Intra-assay Precision (Precision within an assay)

One sample whose concentration between the highest and the second standard were tested twenty times on one plate to assess.

$$CV\% < 8\%$$

Inter-assay Precision (Precision between assays)

One sample whose concentration between the highest and the second standard were tested in thirty-five separate assays to assess

$$CV\% < 10\%$$

RECOVERY

The recovery of PGF2 spiked to three levels throughout the range of the assay in the tissue homogenates and cell culture supernates samples was evaluated.

Sample Type	Average %	Recovery Range
Rat Tissue Homogenates(n =6)	90	84 - 102%

Samples were spiked then diluted prior to assay as directed in Sample Preparation.

PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

