Rat Prostaglandin E2
(PGE2)
ELISA Kit

ZellBio GmbH (Germany)

Cat. No: ZB-10504C-R9648

Samples Type Validated

Serum, Plasma, Cell Lysate, Cell Culture

Supernatant, and Other Related Biological Liquids.

For Research Use Only. Not For In Vitro Diagnosis

Please read this insert thoroughly before using the product.

Important Note:

Only the brochure included in the kit should be referenced for the assay procedure, as it contains the most up-to-date information.

Assay Description

The **ZellBio GmbH** ELISA kit **quantitatively** measures **Rat Prostaglandin E2 (PGE2)** using a Biotin double antibody sandwich technology. Our ELISA kit employs a **one-step** method that minimizes the need for extensive dilution, streamlining the process through advanced laboratory techniques. This kit is intended for research purposes only and is not intended for diagnostic procedures.

Intended Use

The **ZellBio GmbH** Rat Prostaglandin E2 (PGE2) ELISA Kit is designed to quantify Rat PGE2 in rat **serum, plasma, cell lysate, cell culture supernatants**, and other related biological fluids.

Test Principle

The **ZellBio GmbH** assay kit utilizes an enzyme-linked immunosorbent assay (ELISA) based on Biotin double antibody sandwich technology to detect **Rat Prostaglandin E2 (PGE2)**. PGE2 protein is added to wells pre-coated with anti- PGE2 monoclonal antibodies, followed by the addition of biotin-labeled anti- PGE2 antibodies to form an immune complex with streptavidin-HRP. After

incubation and washing to remove unbound enzymes, a substrate is added. The solution will turn blue and change to yellow upon acidification. The color intensity correlates with the concentration of **Rat Prostaglandin E2 (PGE2)**.

Materials supplied in the Test Kit

	Materials	96 Tests	48 Tests
1	Standard (1600pg/mL)	0.3ml	0.3ml
2	Standard diluent	3ml	3ml
3	Microplate	12×8	6×8
4	Strp-HRP-Conjugate Reagent	6ml	3ml
5	Wash Solution (30X)	20ml	20ml
6	Biotin- PGE2 -Ab	1ml	0.5ml
7	Chromogen Solution	11ml	6ml
8	Stop Solution	6ml	3ml
9	Instruction	1	1
10	Adhesive Sheet	2	2

Materials Required but Not Supplied

1. 37°C incubator
2. Standard Microplate reader
3. Precision pipettes and Disposable pipette tips
4. Distilled water
5. Disposable tubes for Standards and samples dilution
6. Absorbent paper

Important Notes

1. Prior to opening the kit, store it at 2-8°C. Allow at least 30 minutes for it to naturally reach room temperature. After breaking the seal of the ELISA coated-plate, store any unused strips in a sealed bag.
2. When adding samples, use a sample injector each time and regularly verify its precision to minimize individual errors.
3. Adhere strictly to the instructions during the reading process with the ELISA reader set according to the standard for accurate experiment results.
4. Avoid cross-contamination by using pipette tips and seal plate membranes only once.
5. Dispose of all samples, washing solutions, and waste materials as biohazardous waste.

6. Store any unused reagents securely. Do not mix reagents from different batches and ensure they are used before their expiration dates.
7. Substrate is sensitive to light and therefore should not be exposed to light for too long" is correct

Washing Procedure

Preparation of 1X Wash Buffer: Dilute the 30X wash buffer with distilled water. If crystals are present in the concentrate, gently mix until fully dissolved.

Manual Washing Method: Hand Washing: Remove liquids from the ELISA plate wells. Place absorbent paper on a flat surface, firmly pat the plate downwards several times. Subsequently, add at least 300µl of diluted wash buffer and allow it to soak for 1-2 minutes. Repeat as necessary.

Automatic Washing Method: Plate Washer Usage: If utilizing an automatic plate washer, ensure familiarity with its operation before use in the assay.

Assay Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle, and high levels of **Rat PGE2** were tested 12 times on a single

plate.

Inter-assay Precision (Precision between assays): 3 samples with low, middle, and high levels of **Rat PGE2** were tested on 3 different plates, with 10 replicates per plate.

The coefficient of variation (CV) was calculated as:

$$CV\ (\%) = (\text{Standard Deviation}/\text{Mean}) \times 100$$

Acceptance Criteria:

Intra-Assay: CV < 10%

Inter-Assay: CV < 12%

Specimen Requirements

1. Samples containing sodium azide (NaN3) must not be tested, as it inhibits the activity of horseradish peroxidase (HRP).
2. After sample collection, extraction should be performed immediately according to relevant protocols. The extracted sample should be tested within 24 hours when stored at 2-8°C. For long-term storage, keep the sample at -20°C or below. Avoid repeated freeze-thaw cycles.
3. **Serum**: Allow the blood to clot for 10-15 minutes at room temperature, then centrifuge at 2000-3000 RPM for 10 minutes. Carefully collect the supernatant. If sediments form during storage, re-centrifuge before use.

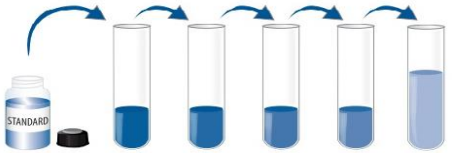
4. **Blood Plasma**: Use EDTA or sodium citrate as the anticoagulant. Mix thoroughly, then centrifuge at 2000-3000 RPM for approximately 10 minutes. Carefully collect the supernatant. If sediments form during storage, re-centrifuge before use.
5. **Cell Culture Supernatant**: Collect the samples in sterile tubes. Centrifuge at 2000-3000 RPM for approximately 10 minutes, then carefully collect the supernatant. For intracellular components, dilute the cell suspension in PBS (pH 7.2-7.4) to a concentration of approximately 1 million cells/mL. Damage the cells through repeated freeze-thaw cycles to release the intracellular contents. Centrifuge at 2000-3000 RPM for approximately 10 minutes, then carefully collect the supernatant.
6. **Solid Tissue Samples**: Weigh the tissue sample and add an appropriate volume of PBS (pH 7.4, 100 mM) as a common buffer. Alternatively, freeze the tissue in liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8°C. Add PBS and homogenize the sample thoroughly by hand or using a homogenizer (100 mg tissue/1 mL buffer). Note: The PBS must contain an anti-protease cocktail to prevent protein degradation. Centrifuge at 4000-6000 RPM for approximately 10 minutes, then carefully collect the supernatant. Aliquot and keep

one for examination, freezing the others for later use. Conduct the experiment immediately or store the sample at 2-8°C for up to 24 hours. For long-term storage, keep the sample at -20°C or below. Avoid repeated freeze-thaw cycles.

Assay procedure

1. **Standard Solution Preparation**: This kit includes a standard solution of original concentration. Users can dilute the standard solution in small tubes according to the instructions provided.

1600pg/mL	Standard No.6	60μl Original Standard
800pg/mL	Standard No.5	60μl Original Standard + 60μl Standard diluents
400pg/mL	Standard No.4	60μl Standard No.5 + 60μl Standard diluents
200pg/mL	Standard No.3	60μl Standard No.4 + 60μl Standard diluent
100pg/mL	Standard No.2	60μl Standard No.3 + 60μl Standard diluent
50pg/mL	Standard No.1	60μl Standard No.2 + 60μl Standard diluent
0pg/mL	Standard No.0	60μl Standard diluent



pg/mL	1600	800	400	200	100	50

2. **Determining Stripes and Standards**: The number of stripes required is based on the samples and standards to be tested. It is recommended to include duplicates for each standard solution and the blank well.
3. **Sample Injection**: a) Blank Well: Exclude sample and add anti-PGE2 antibody labeled with biotin or streptavidin-HRP to the comparison blank well, following the same steps without Chromogen solution and stop solution. b) Standard Solution Well: Add 50µl standard and 50µl streptavidin-HRP (biotin antibodies pre-bound in the standard, no additional biotin antibodies required). c) Sample Well: Add 40µl sample, followed by 10µl PGE2 antibodies and 50µl streptavidin-HRP. Seal with a plate membrane, gently mix, and incubate at 37°C for 60 minutes.
4. **Preparation of Washing Solution**: Dilute the washing concentration (30X) with distilled water for future use.
5. **Washing Procedure**: Carefully remove the seal plate membrane, drain excess liquid, and shake off any remaining liquid. Fill each well with the washing solution, drain after 30 seconds, repeat four times, and blot the plate.

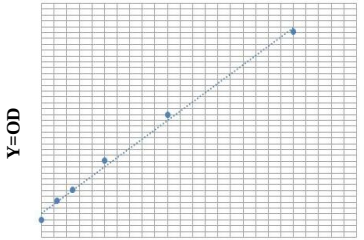
6. **Color Development**: Add 100µl Chromogen solution to each well and incubate at 37°C away from light for 10-20 minutes for color development.
7. **Stop Reaction**: Add 50µl Stop Solution to each well to halt the reaction (blue color changes to yellow immediately).
8. **Reading Absorbance**: Use a blank well as zero reference. Measure the absorbance (OD) of each well individually at 450 nm wavelength within 10 minutes of adding the stop solution.
9. **Standard Curve and Calculation**: Generate a standard curve using point-to-point calculation mode based on standards' concentrations and corresponding OD values. Calculate sample concentrations based on their OD values. Specialized software can also be utilized for accurate calculations. By following these steps meticulously, the assay can be conducted effectively, ensuring accurate results and reliable data interpretation.

Calculation

Plotting the Standard Curve: Use the concentration of standards on the x-axis and the OD (Optical Density) values on the y-axis. Plot the standard curve on graph paper.

Determining Sample Concentration: Locate the corresponding concentration of the sample based on its OD value by referencing the

standard curve. Utilize point-to-point calculation or calculate the linear regression equation of the standard curve using the concentrations of the standards and their OD values. Substitute the OD value of the sample into the linear regression equation or the standard curve to determine its concentration accurately. By following these steps, you can effectively analyze the data obtained from the assay, determine the concentration of the samples, and ensure the reliability of your results.



X= Rat PGE2 Concentration

A typical standard curve of ZellBio GmbH **Rat PGE2** Assay kit
(The chart is for illustrative purposes only)

Assay range：50pg/mL →1600pg/mL

Sensitivity：7pg/mL

Assay Summary

1. Prepare Reagents, Samples, and Standards
2. Sample and Standard Addition:
   - Add 40µl sample + 10µl PGE2 Antibody
   - Add 50µl Standards
   - Add 50µl Streptavidin-HRP
   - Incubate for 60 minutes at 37°C
3. Washing:
   - Wash the plate 5 times with 300µl diluted wash buffer
4. Color Development:
   - Add 100µl Chromogen Solution
   - Incubate for 10-20 minutes at 37°C
5. Stop Reaction:
   - Add 50µl Stop Solution
6. Absorbance Measurement:
   - Read the OD value within 10 minutes at 450 nm
7. Calculation:
   - Determine sample concentrations using the standard curve

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