Instructions for Use

For quantitative detection of Prostaglandin E2 (PGE$_2$) in serum, saliva, urine and tissue culture media

This product is for research use only and is not intended for diagnostic use.
Table of Contents

INTRODUCTION
1. BACKGROUND 3
2. ASSAY SUMMARY 4

GENERAL INFORMATION
3. PRECAUTIONS 5
4. STORAGE AND STABILITY 6
5. MATERIALS SUPPLIED 6
6. MATERIALS REQUIRED, NOT SUPPLIED 7
7. LIMITATIONS 7
8. TECHNICAL HINTS 8

ASSAY PREPARATION
9. REAGENT PREPARATION 9
10. STANDARD PREPARATIONS 10
11. SAMPLE COLLECTION AND STORAGE 12
12. PLATE PREPARATION 14

ASSAY PROCEDURE
13. ASSAY PROCEDURE 15

DATA ANALYSIS
14. CALCULATIONS 17
15. TYPICAL DATA 18
16. TYPICAL SAMPLE VALUES 20
17. ASSAY SPECIFICITY 22

RESOURCES
18. TROUBLESHOOTING 23
19. NOTES 24
INTRODUCTION

1. BACKGROUND

Abcam’s Prostaglandin E2 (PGE$_2$) *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Prostaglandin E2 in serum, saliva, urine and tissue culture media and other biological fluids.

A mouse IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-Prostaglandin E2 antibody. After incubation the excess reagents are washed away and pNpp substrate is added and is catalyzed by AP to produce a yellow color. The intensity of the yellow coloration is inversely proportional to the amount of Prostaglandin E2 captured in the plate.

Prostaglandin E2 is formed in a variety of cells from PGH2, which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthetase. Prostaglandin E2 has been shown to have a number of biological actions, including vasodilation, both anti- and proinflammatory action, modulation of sleep/wake cycles, and facilitation of the replication of human immunodeficiency virus. It elevates cAMP levels, stimulates bone resorption, and has thermoregulatory effects. It has been shown to be a regulator of sodium excretion and renal hemodynamics.
2. **ASSAY SUMMARY**

Prepare all reagents and samples as instructed.

Add standards and samples to appropriate wells.

Add prepared labeled AP-conjugate to appropriate wells.

Add PGE$_2$ antibody to appropriate wells. Incubate at room temperature.

Add pNpp substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

- Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.
- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.
- The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg$^{2+}$ and Zn$^{2+}$ ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.
- We test the performance of this kit with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
- The Prostaglandin E2 Standard provided, is supplied in ethanolic buffer at a pH optimized to maintain PGE$_2$ integrity. Care should be taken handling this material because of the known and unknown effects of prostaglandin.
4. **STORAGE AND STABILITY**

Store kit at 4°C immediately upon receipt, apart from the AP Conjugate & Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles.

Refer to list of materials supplied for storage conditions of individual components.

5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat anti-mouse IgG Microplate (12 x 8 wells)</td>
<td>96 Wells</td>
<td>2-8°C</td>
</tr>
<tr>
<td>PEG₂ Alkaline Phosphatase Conjugate</td>
<td>6 mL</td>
<td>-20°C</td>
</tr>
<tr>
<td>PEG₂ Antibody</td>
<td>6 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Prostaglandin E2 Standard</td>
<td>500 µL</td>
<td>-20°C</td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>30 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>20X Wash Buffer Concentrate</td>
<td>30 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>pNpp Substrate</td>
<td>20 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>5 mL</td>
<td>2-8°C</td>
</tr>
</tbody>
</table>
6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- 200 mg C18 Reverse Phase Extraction Columns (only required for extraction of samples containing low levels of \( \text{PGE}_2 \))
- 2M hydrochloric acid (only required for extraction of samples containing low levels of \( \text{PGE}_2 \))
- Deionized water
- Ethanol
- Hexane
- Ethyl acetate

7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent
- Pipette standards and samples to the bottom of the wells
- Add the reagents to the side of the well to avoid contamination
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results
- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions
9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 **PGE$_2$ Alkaline Phosphatase Conjugate**

Allow the Prostaglandin E2 Alkaline Phosphatase Conjugate to equilibrate to room temperature. Any unused conjugate should be aliquoted and stored at --20°C.

9.2 **1X Wash Buffer**

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently. 1X Wash buffer can be stored at room temperature for three months, or until the expiration date of the kit, whichever is earlier.
10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every experiment. Diluted standards should be used within 60 minutes of preparation.

10.1 Allow the 50,000 pg/mL PGE$_2$ **Stock Standard** solution to warm to room temperature.

Any unused Stock Standard solution should be stored at -20°C.

10.2 Label eight tubes with numbers 1 - 8.

10.3 Add 500 μL appropriate sample diluent into tubes numbers 2 – 8:

For **plasma, serum and urine** samples use Assay Buffer to dilute the standard.

For **cell culture supernatant** samples use tissue culture media to dilute the standard.

10.4 Prepare a 2,500 pg/mL **Standard 1** by adding 50 μL of the 50,000 pg/mL Stock Standard to 950 μL to tube 1. Mix thoroughly and gently.

10.5 Prepare **Standard 2** by transferring 500 μL from Standard 1 to tube 2. Mix thoroughly and gently.

10.6 Prepare **Standard 3** by transferring 500 μL from Standard 2 to tube 3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes 4 through 7.

10.8 **Standard 8** contains no protein and is the 0 pg/mL (Bo) control.
<table>
<thead>
<tr>
<th>Standard #</th>
<th>Sample to Dilute</th>
<th>Volume to Dilute (µL)</th>
<th>Volume of Diluent (µL)</th>
<th>Starting Conc. (pg/mL)</th>
<th>Final Conc. (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stock</td>
<td>50</td>
<td>950</td>
<td>50,000</td>
<td>2,500</td>
</tr>
<tr>
<td>2</td>
<td>Standard 1</td>
<td>500</td>
<td>500</td>
<td>2,500</td>
<td>1,250</td>
</tr>
<tr>
<td>3</td>
<td>Standard 2</td>
<td>500</td>
<td>500</td>
<td>1,250</td>
<td>625</td>
</tr>
<tr>
<td>4</td>
<td>Standard 3</td>
<td>500</td>
<td>500</td>
<td>625</td>
<td>313</td>
</tr>
<tr>
<td>5</td>
<td>Standard 4</td>
<td>500</td>
<td>500</td>
<td>313</td>
<td>156</td>
</tr>
<tr>
<td>6</td>
<td>Standard 5</td>
<td>500</td>
<td>500</td>
<td>156</td>
<td>78.1</td>
</tr>
<tr>
<td>7</td>
<td>Standard 6</td>
<td>500</td>
<td>500</td>
<td>78.1</td>
<td>39.1</td>
</tr>
<tr>
<td>8 B₀</td>
<td>None</td>
<td>-</td>
<td>500</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>
11. SAMPLE COLLECTION AND STORAGE

- The PGE$_2$ kit is compatible with PGE$_2$ samples in a wide range of matrices after dilution in Assay Buffer. However, the end user must verify that the recommended dilutions are appropriate for their samples. Samples containing mouse IgG may interfere with the assay.

- Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or Assay Buffer to calculate concentrations of PEG$_2$ in the appropriate matrix.

- For tissue, urine and plasma samples, prostaglandin synthetase inhibitors such as indomethacin or meclofenamic acid at concentrations up to 10 $\mu$g/mL should be added to either the tissue homogenate or urine and plasma samples. Some samples normally have very low levels of PGE$_2$ present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

11.1. Acidify the plasma, urine or tissue homogenate by addition of 2M HCl to pH of 3.5. Approximately 50 $\mu$L of HCl will be needed per mL of plasma. Allow to sit at 4°C for 15 minutes. Centrifuge samples in a micro-centrifuge for 2 minutes to remove any precipitate.

11.2. Prepare the C18 reverse phase column (see Section 6) by washing with 10 mL of ethanol followed by 10 mL of deionized water.

11.3. Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5 mL/minute. Wash the column with 10 mL of water, followed by 10 mL of 15% ethanol, and
finally 10 mL hexane. Elute the sample from the column by addition of 10 mL ethyl acetate.

11.4. If analysis is to be carried out immediately, evaporate samples under a stream of nitrogen. Add at least 250 μL of Assay Buffer to the dried sample. Mix well and hold at room temperature for 5 minutes. Repeat twice more. If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at -80°C until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running assay and reconstitute as above.

- 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.
12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

**Recommended plate layout**

<table>
<thead>
<tr>
<th>A1-2</th>
<th>Standard #1</th>
<th>A3-4</th>
<th>Blank Wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1-2</td>
<td>Standard #2</td>
<td>B3-4</td>
<td>Total Activity Wells (TA)</td>
</tr>
<tr>
<td>C1-2</td>
<td>Standard #3</td>
<td>C3-4</td>
<td>Non-Specific Binding Wells (NSB)</td>
</tr>
<tr>
<td>D1-2</td>
<td>Standard #4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1-2</td>
<td>Standard #5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1-2</td>
<td>Standard #6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1-2</td>
<td>Standard #7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1-2</td>
<td>Standard #8 (Bo)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
13. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use
- It is recommended to assay all standards, controls and samples in duplicate
- Refer to the recommended plate layout in Section 12 before proceeding with the assay.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Add 100 µL appropriate standard diluent (Assay Buffer or tissue culture media) into the NSB wells.

13.3 Add 100 µL of prepared standards and diluted samples to appropriate wells.

13.4 Add 50 µL of Assay Buffer into the NSB wells.

13.5 Add 50 µL of PEG₂ Alkaline Phosphatase Conjugate into each well, except the Total Activity (TA) and Blank wells.

13.6 Add 50 µL of PEG₂ Antibody into each well, except the Blank, TA and NSB wells.

*Note:* Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.

13.7 Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided.

13.8 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 Washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.9 Add 5 µL of the PEG₂ Alkaline Phosphatase Conjugate to the TA wells.
13.10 Add 200 μL of the pNpp Substrate solution to every well. Incubate at room temperature for 45 minutes without shaking.

13.11 Add 50 μL Stop Solution into each well. The plate should be read immediately.

13.12 Blank the plate reader against the blank wells, read the O.D. absorbance at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the blank wells, manually subtract the mean optical density of the blank wells from all readings.
14. CALCULATIONS

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

   \[ \text{Average Net OD} = \text{Average Bound OD} - \text{Average NSB OD} \]

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

   \[ \text{Percent Bound} = \left( \frac{\text{Net OD}}{\text{Net Bo OD}} \right) \times 100 \]

3. Plot both the Percent Bound and the Net OD versus Concentration of PGE\(_2\) for the standards. Sample concentrations may be calculated off of Net OD values using the desired curve fitting.

Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
15. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

---

**Sample** | **Mean OD (-Blank)** | **% Bound** | **Percent (pg/mL)**
--- | --- | --- | ---
Blank | 0.087 | | |
TA | 1.22 | | |
NSB | 0.002 | 0 | |
S8 (Bo) | 0.982 | 100 | 0
S1 | 0.074 | 7.3 | 2,500
S2 | 0.121 | 12.1 | 1,250
S3 | 0.205 | 20.7 | 625
S4 | 0.337 | 34.2 | 313
S5 | 0.507 | 51.5 | 156
S6 | 0.663 | 67.4 | 78.1
S7 | 0.804 | 81.8 | 39.1
Unknown 1 | 0.299 | 30.30 | 375.3
Unknown 2 | 0.61 | 62.00 | 100.7
TYPICAL QUALITY CONTROL PARAMETERS

Total Activity Added = 1.220 x 10 = 12.20

%NSB = 0.02%

%Bo/TA = 8.04%

Quality of Fit = 1.000 (Calculated from 4 parameter logistic curve fit)

20% Intercept = 664 pg/mL

50% Intercept = 162 pg/mL

80% Intercept = 43 pg/mL
16. TYPICAL SAMPLE VALUES

SENSITIVITY –
The sensitivity, minimum detectable dose of PGE\textsubscript{2} using this Abcam ELISA kit was found to be 13.4 pg/mL. This was determined by the average optical density of the 0 pg/mL Standard and comparing to the average optical density for Standard 7. The detection limit was determined as the concentration of PGE\textsubscript{2} measured at two standard deviations from the zero along the standard curve.

SAMPLE RECOVERY –
Recovery was determined by PGE\textsubscript{2} into tissue culture media, Human saliva, serum, and urine. Mean recoveries are as follows:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Recommended Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Culture Media</td>
<td>104.4</td>
<td>None</td>
</tr>
<tr>
<td>Human Saliva</td>
<td>123.3</td>
<td>1:10</td>
</tr>
<tr>
<td>Human Urine</td>
<td>108.9</td>
<td>1:10</td>
</tr>
<tr>
<td>Human Male Serum</td>
<td>126.1</td>
<td>1:10</td>
</tr>
<tr>
<td>Human Female Serum</td>
<td>113.7</td>
<td>1:10</td>
</tr>
<tr>
<td>Human Whole Blood</td>
<td>101.2</td>
<td>1:10</td>
</tr>
</tbody>
</table>

LINEARITY OF DILUTION –
A sample containing 50,000 pg/mL PGE\textsubscript{2} was diluted 6 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual PGE\textsubscript{2} concentration versus measured PGE\textsubscript{2} concentration.

The line obtained had a slope of 1.1610 and a correlation coefficient of 1.000.
**DATA ANALYSIS**

**PRECISION –**

**Intra-Assay**

<table>
<thead>
<tr>
<th></th>
<th>PGE&lt;sub&gt;2&lt;/sub&gt;(pg/mL)</th>
<th>Intra-Assay %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>116</td>
<td>8.9</td>
</tr>
<tr>
<td>Medium</td>
<td>492</td>
<td>5.8</td>
</tr>
<tr>
<td>High</td>
<td>2,416</td>
<td>17.5</td>
</tr>
</tbody>
</table>

**Inter-Assay**

<table>
<thead>
<tr>
<th></th>
<th>PGE&lt;sub&gt;2&lt;/sub&gt; (pg/mL)</th>
<th>Inter-Assay %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>111</td>
<td>3.0</td>
</tr>
<tr>
<td>Medium</td>
<td>419</td>
<td>5.1</td>
</tr>
<tr>
<td>High</td>
<td>1,902</td>
<td>3.9</td>
</tr>
</tbody>
</table>
### 17. ASSAY SPECIFICITY

The cross reaction of the antibody calculated at 50% is:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross Reaction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PGE$_2$</strong></td>
<td>100 %</td>
</tr>
<tr>
<td><strong>PGE$_1$</strong></td>
<td>70 %</td>
</tr>
<tr>
<td><strong>PGE$_3$</strong></td>
<td>16.3 %</td>
</tr>
<tr>
<td><strong>PGF$_1$ alpha</strong></td>
<td>1.4 %</td>
</tr>
<tr>
<td><strong>PGF$_2$ alpha</strong></td>
<td>0.7 %</td>
</tr>
<tr>
<td><strong>6-keto-PGF$_1$ alpha</strong></td>
<td>0.6 %</td>
</tr>
<tr>
<td><strong>PGA$_2$</strong></td>
<td>0.1 %</td>
</tr>
<tr>
<td><strong>PGB$_1$</strong></td>
<td>0.1 %</td>
</tr>
<tr>
<td><strong>13,14-dihydro-15-keto-PGF$_2$ alpha</strong></td>
<td>&lt;0.1 %</td>
</tr>
<tr>
<td><strong>6,15-keto-13,14-dihydro-PGF$_1$ alpha</strong></td>
<td>&lt;0.1 %</td>
</tr>
<tr>
<td><strong>Thromboxane B$_2$</strong></td>
<td>&lt;0.1 %</td>
</tr>
<tr>
<td><strong>2-Arachidonoylglycerol</strong></td>
<td>&lt;0.1 %</td>
</tr>
<tr>
<td><strong>Anandamide</strong></td>
<td>&lt;0.1 %</td>
</tr>
<tr>
<td><strong>PGD$_2$</strong></td>
<td>&lt;0.1 %</td>
</tr>
<tr>
<td><strong>Arachadonic Acid</strong></td>
<td>&lt;0.1 %</td>
</tr>
</tbody>
</table>
# 18. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standards dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td>Low Signal</td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; change to overnight standard/sample incubation</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td>Samples give higher value than the highest standard</td>
<td>Starting sample concentration is too high.</td>
<td>Dilute the specimens and repeat the assay</td>
</tr>
<tr>
<td>Large CV</td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the kit</td>
<td>Store the all components as directed.</td>
</tr>
</tbody>
</table>
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