ab133041 – PGF$_2$alpha ELISA Kit

Instructions for Use

For quantitative detection of PGF$_2$ alpha in Porcine Plasma, Porcine and Human 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  
2. ASSAY SUMMARY  

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

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

## ASSAY PROCEDURE
13. ASSAY PROCEDURE  

## DATA ANALYSIS
14. CALCULATIONS  
15. TYPICAL DATA  
16. TYPICAL SAMPLE VALUES  
17. ASSAY SPECIFICITY  

## RESOURCES
18. TROUBLESHOOTING  
19. NOTES
1. BACKGROUND

Abcam’s PGF$_2$ alpha *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of PGF$_2$ alpha in Porcine Plasma, Porcine and Human Serum, Saliva, Urine and Tissue Culture Media.

A donkey anti-sheep 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-PGF$_2$ alpha antigen and a polyclonal sheep antibody specific to PGF$_2$ alpha. After incubation the excess reagents are washed away. pNpp substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the yellow coloration is inversely proportional to the amount of PGF$_2$ alpha captured in the plate.

Prostaglandin F$_2$ alpha (PGF$_2$ alpha) is formed in a variety of cells from PGH$_2$, which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthetase. PGF$_2$ alpha is often viewed as an antagonist to PGF$_2$ alpha due to their opposing effects on various tissues. PGF$_2$ alpha is a potent bronchoconstrictor and has been implicated in asthma attacks. PGF$_2$ alpha is also involved in reproductive functions including corpus luteum regulation, uterine contractions, and sperm motility. This has led to its use in terminating pregnancies and inducing labor at term. High levels of PGF$_2$ alpha have also been associated with pre-eclampsia.
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 PGF$_2$ alpha 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 this kit’s performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
- The PGF\(_{2\alpha}\) alpha Standard provided is supplied in ethanolic buffer at a pH optimized to maintain PGF\(_{2\alpha}\) alpha 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 Alkaline Phosphatase Conjugate and 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</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donkey anti-sheep IgG Microplate (12 x 8 wells)</td>
<td>96 Wells</td>
<td>4°C</td>
</tr>
<tr>
<td>PGF$_2$ alpha Alkaline Phosphatase Conjugate</td>
<td>5 mL</td>
<td>-20°C</td>
</tr>
<tr>
<td>PGF$_2$ alpha Antibody</td>
<td>5 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>PGF$_2$ alpha Standard</td>
<td>500 µL</td>
<td>-20°C</td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>30 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>20X Wash Buffer Concentrate</td>
<td>30 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>pNpp Substrate</td>
<td>20 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>5 mL</td>
<td>4°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 PGF$_2$ alpha)
- 2M hydrochloric acid (only required for extraction of samples containing low levels of PGF$_2$ alpha)
- 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 **PGF$_2$ alpha Alkaline Phosphatase Conjugate**

Allow the PGF$_2$ alpha Alkaline Phosphatase Conjugate to equilibrate to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -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.
10. **STANDARD PREPARATIONS**

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

10.1 Allow the 500,000 pg/mL PGF$_2$ alpha **Stock Standard** solution to equilibrate to room temperature. The standard solution should be stored at -20°C. Avoid repeated freeze-thaw cycles.

10.2 Label nine tubes with numbers 1 – 8 and one with B$_o$.

10.3 Prepare a 50,000 pg/mL **Standard 1** by adding 100 µL of the 500,000 pg/mL Stock Standard 900 µL of the appropriate diluent to tube 1. Mix thoroughly and gently.

10.4 Add 750 µL of the appropriate diluent to tubes 2 – 8.

10.5 Prepare **Standard 2** by transferring 250 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.6 Prepare **Standard 3** by transferring 250 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 8.
### ASSAY PREPARATION

<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>Standard</td>
<td>100</td>
<td>900</td>
<td>500,000</td>
<td>50,000</td>
</tr>
<tr>
<td>2</td>
<td>Standard 1</td>
<td>250</td>
<td>750</td>
<td>50,000</td>
<td>12,500</td>
</tr>
<tr>
<td>3</td>
<td>Standard 2</td>
<td>250</td>
<td>750</td>
<td>12,500</td>
<td>3,125</td>
</tr>
<tr>
<td>4</td>
<td>Standard 3</td>
<td>250</td>
<td>750</td>
<td>3,125</td>
<td>781.25</td>
</tr>
<tr>
<td>5</td>
<td>Standard 4</td>
<td>250</td>
<td>750</td>
<td>781.25</td>
<td>195.31</td>
</tr>
<tr>
<td>6</td>
<td>Standard 5</td>
<td>250</td>
<td>750</td>
<td>195.31</td>
<td>48.83</td>
</tr>
<tr>
<td>7</td>
<td>Standard 6</td>
<td>250</td>
<td>750</td>
<td>48.83</td>
<td>12.2</td>
</tr>
<tr>
<td>8</td>
<td>Standard 7</td>
<td>250</td>
<td>750</td>
<td>12.2</td>
<td>3.05</td>
</tr>
<tr>
<td>Bo</td>
<td>None</td>
<td>-</td>
<td>750</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

![Diagram of assay preparation](image)
ASSAY PREPARATION

11. SAMPLE COLLECTION AND STORAGE

- The PGF$_2$ alpha kit is compatible with PGF$_2$ alpha 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.

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

11.1. Materials Needed

11.1.1. PGF$_2$ alpha Standard to allow extraction efficiency to be accurately determined.

11.1.2. 2M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate.

11.1.3. 200 mg C18 Reverse Phase Extraction Columns.

11.2. Procedure.

11.2.1. Acidify the plasma, urine or tissue homogenate by addition of 2M HCl to pH of 3.5. Approximately 50 μL of HCl will be needed per mL of plasma. Allow to sit at 4 °C for 15 minutes. Centrifuge samples in a microcentrifuge for 2 minutes to remove any precipitate.
11.2.2. Prepare the C18 reverse phase column by washing with 10 mL of ethanol followed by 10 mL of deionized water.

11.2.3. Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5mL/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.

11.2.4. 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 samples. Vortex well, then allow to sit five minutes at room temperature. 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.
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 each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- 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.
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
- **Two wells must be used to measure Blank Activity ($B_0$) and Total Activity (TA).**

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

13.2 Add 100 μL of the samples, standards and $B_0$ to the appropriate wells.

13.3 Add 50 μL of PGF$_2$ alpha Alkaline Phosphatase Conjugate into each well, except the TA wells.

13.4 Add 50 μL of PGF$_2$ alpha antibody into each well, except the TA wells.

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

13.5 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.

13.6 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.

13.7 Add 5 μL of the PGF$_{2α}$ Alkaline Phosphatase Conjugate to the TA wells.

13.8 Add 200 μL of the pNpp Substrate solution to every well. Incubate at room temperature for 45 minutes without shaking.

13.9 Add 50 μL Stop Solution into each well.

13.10 Immediately read the O.D. absorbance at 405 nm, preferably with correction between 570 and 590 nm.
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). Values should be expressed as a percentage of the B₀. Divide the replicate average sample or standard OD measurement by the replicate average B₀ measurement. Interpolate protein concentrations for unknown samples from the standard curve plotted. 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.

- Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD bound:

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

- Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (B₀), using the following formula:

  \[
  \text{Percent Bound} = \left( \frac{\text{Net OD}}{\text{Net B₀ OD}} \right) \times 100
  \]

- Plot both the Percent Bound and the Net OD versus Concentration of PGF₂ alpha for the standards. Sample concentrations may be calculated off of Net OD values using the desired curve fitting
15. TYPICAL DATA

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean OD (-Blank)</th>
<th>% Bound</th>
<th>PGF$_2$ alpha pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>1.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard 1</td>
<td>0.020</td>
<td>2.9</td>
<td>50,000</td>
</tr>
<tr>
<td>Standard 2</td>
<td>0.050</td>
<td>7.4</td>
<td>12,500</td>
</tr>
<tr>
<td>Standard 3</td>
<td>0.121</td>
<td>17.7</td>
<td>3,125</td>
</tr>
<tr>
<td>Standard 4</td>
<td>0.220</td>
<td>32.1</td>
<td>781</td>
</tr>
<tr>
<td>Standard 5</td>
<td>0.347</td>
<td>50.5</td>
<td>195</td>
</tr>
<tr>
<td>Standard 6</td>
<td>0.516</td>
<td>74.9</td>
<td>48.8</td>
</tr>
<tr>
<td>Standard 7</td>
<td>0.642</td>
<td>93.2</td>
<td>12.2</td>
</tr>
<tr>
<td>Standard 8</td>
<td>0.680</td>
<td>98.7</td>
<td>3.05</td>
</tr>
<tr>
<td>B$_o$</td>
<td>0.689</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Unknown 1</td>
<td>0.507</td>
<td>73.6</td>
<td>182</td>
</tr>
<tr>
<td>Unknown 2</td>
<td>0.110</td>
<td>16.0</td>
<td>5,852</td>
</tr>
</tbody>
</table>
**Typical Quality Control Parameters**

- **Total Activity Added** = $1.021 \times 10 = 10.21$
- **%Bo/TA** = 6.70%
- **Quality of Fit** = 0.9999 (Calculated from 4 parameter logistic curve fit)
- **20% Intercept** = 2,041 pg/mL
- **50% Intercept** = 230 pg/mL
- **80% Intercept** = 33 pg/mL
16. TYPICAL SAMPLE VALUES

SENSITIVITY –
Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as B₀, and comparing to the average optical density for sixteen (16) wells run with Standard #8. The detection limit was determined as the concentration of PGF₂α measured at two (2) standard deviations from the zero along the standard curve and was determined to be 6.71 pg/mL.

SAMPLE RECOVERY –
Recovery was determined by PGF₂α 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>-</td>
<td>Neat</td>
</tr>
<tr>
<td>Human Saliva</td>
<td>103.5</td>
<td>≥1:20</td>
</tr>
<tr>
<td>Human Urine</td>
<td>107.0</td>
<td>≥1:20</td>
</tr>
<tr>
<td>Porcine &amp; Human Serum</td>
<td>100.3</td>
<td>≥1:10</td>
</tr>
<tr>
<td>Porcine Plasma</td>
<td>96.9</td>
<td>≥1:10</td>
</tr>
</tbody>
</table>

LINEARITY OF DILUTION –
A sample containing 10,000 pg/mL PGF₂α was diluted 4 times 1:10 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual PGF₂α concentration versus measured PGF₂α concentration. The line obtained had a slope of 1.069 and a correlation coefficient of 0.999.
DATA ANALYSIS

PRECISION –

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of PGF$_{2\alpha}$ and running these samples multiple times (n=10) in the same assay. Inter-assay precision was determined by measuring two samples with low and high concentrations of PGF$_{2\alpha}$ in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of PGF$_{2\alpha}$ determined in these assays as calculated by a 4 parameter logistic curve fitting program.

<table>
<thead>
<tr>
<th></th>
<th>PGF$_{2\alpha}$ (pg/mL)</th>
<th>Intra-Assay %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>83</td>
<td>13.1</td>
</tr>
<tr>
<td>Medium</td>
<td>405</td>
<td>6.8</td>
</tr>
<tr>
<td>High</td>
<td>916</td>
<td>4.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>PGF$_{2\alpha}$ (pg/mL)</th>
<th>Inter-Assay %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>47</td>
<td>9.7</td>
</tr>
<tr>
<td>Medium</td>
<td>290</td>
<td>5.5</td>
</tr>
<tr>
<td>High</td>
<td>731</td>
<td>3.1</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>PGF$_2$ alpha</td>
<td>100 %</td>
</tr>
<tr>
<td>PGF$_1$ alpha</td>
<td>11.82 %</td>
</tr>
<tr>
<td>PGD$_2$</td>
<td>3.62 %</td>
</tr>
<tr>
<td>6-keto-PGF$_1$ alpha</td>
<td>1.38 %</td>
</tr>
<tr>
<td>PGI$_2$</td>
<td>1.25 %</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>0.77 %</td>
</tr>
<tr>
<td>Thromboxane B2</td>
<td>0.77 %</td>
</tr>
<tr>
<td>8-iso PGF$_2$ alpha</td>
<td>0.73 %</td>
</tr>
<tr>
<td>PGE$_1$</td>
<td>0.39 %</td>
</tr>
<tr>
<td>PGA$_2$</td>
<td>&lt;0.10 %</td>
</tr>
<tr>
<td>6,15-keto-13,14-dihydro- PGF$_2$ alpha</td>
<td>&lt;0.01 %</td>
</tr>
<tr>
<td>2-Arachidonoylglycerol</td>
<td>&lt;0.01 %</td>
</tr>
<tr>
<td>Anandamide</td>
<td>&lt;0.01 %</td>
</tr>
</tbody>
</table>
## 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>
19. NOTES
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