**PROSTAGLANDIN E2 KITS**

**PROTOCOL**

**Part # 62P2APEG & 62P2APEH**

**Test size#:** 500 tests (62P2APEG), 10,000 tests (62P2APEH) - assay volume: 20 µL

**Revision:** 02-Jan.2018

**Store at:** 2-8°C (62P2APEG); 2-8°C (62P2APEH)

For research use only. Not for use in diagnostic procedures.

**ASSAY PRINCIPLE**

Cisbio Bioassays’ Prostaglandin E2 assay is only intended for quantitative measurement of Prostaglandin E2 in buffered solution or in cell culture supernatants, using HTRF® technology.

Native Prostaglandin E2 produced by cells is detected in a competitive assay format using a specific antibody labeled with Europium Cryptate (donor) and Prostaglandin E2 labelled with d2 (acceptor). The detection principle is based on HTRF® technology. When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). The Prostaglandin E2 present in the sample competes with the binding between the two conjugates and thereby prevents FRET from occurring. The specific signal is inversely proportional to the Prostaglandin E2 concentration (Fig. 1).

![Figure 1: Principle of HTRF® Prostaglandin E2 competitive assay.](image)

**PROTOCOL AT A GLANCE**

<table>
<thead>
<tr>
<th>ADD</th>
<th>READ</th>
<th>ANALYSE</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Prostaglandin E2-acceptor" /> Prostaglandin E2-acceptor</td>
<td><img src="image" alt="Fluorescence Resonance Energy Transfer" /></td>
<td><img src="image" alt="HTRF Signal" /></td>
</tr>
</tbody>
</table>

**PROTOCOL AT A GLANCE**

**ADD**

- 10 µL Standard or Sample
- 5 µL Prostaglandin E2 acceptor
- 5 µL Anti-Prostaglandin E2 donor Antibody

**READ**

- Incubate 5 hours @ RT or overnight at 4°C

**ANALYSE**

- HTRF Signal

Make sure you use the appropriate setup for Eu³⁺ Cryptate. For more information about set-up and HTRF® compatible readers, please visit our website at: [http://www.cisbio.com/compatible-readers](http://www.cisbio.com/compatible-readers)
MATERIALS PROVIDED:

<table>
<thead>
<tr>
<th>Kit components</th>
<th>500 tests *</th>
<th>10,000 tests *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cat # 62P2APEG</td>
<td>Cat # 62P2APEH</td>
</tr>
<tr>
<td>Prostaglandin E2 Standard</td>
<td>1 vial Lyophilized</td>
<td>1 vial Lyophilized</td>
</tr>
<tr>
<td>Concentrated PGE2 Ref# 62PG2CDA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Prostaglandin E2-Eu** Cryptate Antibody</td>
<td>1 vial Lyophilized</td>
<td>1 vial Lyophilized</td>
</tr>
<tr>
<td>Prostaglandin E2-d2</td>
<td>1 vial Lyophilized</td>
<td>1 vial Lyophilized</td>
</tr>
<tr>
<td>Diluent ** ready to use</td>
<td>1 vial 20 mL (62DL2DDD)</td>
<td>1 vial 20 mL (62DL2DDD)</td>
</tr>
<tr>
<td>Detection buffer *** ready to use</td>
<td>1 vial 7 mL Detection Buffer# 2</td>
<td>1 vial 105 mL Detection Buffer# 3</td>
</tr>
</tbody>
</table>

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 and 10,000 tests respectively in 20 µL final.
** Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.
*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

PURCHASE SEPARATELY:
- HTRF® 96-well low volume plate Ref# 66PL96001 *
- HTRF® 384-well low volume plate Ref# 66PL384025 *
- HTRF®-Certified Reader **. Make sure the setup for Eu³⁺ Cryptate is used.
- Use white plate only.

* For HTRF microplate recommendations, please visit http://www.cisbio.com/drug-discovery/htrf-microplate-recommendations
** For a list of HTRF-compatible readers and setup recommendations, please visit http://www.cisbio.com/compatible-readers

STORAGE AND STABILITY

Store the kit at 2-8°C. Under proper storage conditions, reagents are stable until the expiry date indicated on the label.

If lyophilized, once reconstituted, antibodies and standard stock solutions may be frozen. They can be thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.

Volume of Prostaglandin E2 standard aliquots should not be under 20 µL.

REAGENT PREPARATION

BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Before use, allow diluent and buffer to warm up at room temperature and homogenize them with a vortex.
- Prostaglandin E2 standards (for standard curve) must be prepared in diluent or in the same medium as the samples.

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.
## TO PREPARE STANDARD & DETECTION REAGENTS STOCK SOLUTIONS:

<table>
<thead>
<tr>
<th>500 TESTS KIT - 62P2APEG</th>
<th>10,000 TESTS KIT - 62P2APEH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-Prostaglandin E2-Eu&lt;sup&gt;3+&lt;/sup&gt; Cryptate antibody</strong></td>
<td><strong>Anti-Prostaglandin E2-Eu&lt;sup&gt;3+&lt;/sup&gt; Cryptate antibody</strong></td>
</tr>
<tr>
<td>Reconstitute the Anti-Prostaglandin E2-Eu&lt;sup&gt;3+&lt;/sup&gt; Cryptate antibody with 2.5 mL detection buffer #2. Mix gently. This ready to use 1 X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.</td>
<td>Reconstitute the Anti-Prostaglandin E2-Eu&lt;sup&gt;3+&lt;/sup&gt; Cryptate antibody with 2.5 mL distilled water. Mix gently. This 20 X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.</td>
</tr>
<tr>
<td><strong>Prostaglandin E2-d2</strong></td>
<td><strong>Prostaglandin E2-d2</strong></td>
</tr>
<tr>
<td>Reconstitute the Prostaglandin E2-d2 with 2.5 mL detection buffer #2. Mix gently. This ready to use 1 X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.</td>
<td>Reconstitute the Prostaglandin E2-d2 with 2.5 mL distilled water. Mix gently. This 20 X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.</td>
</tr>
<tr>
<td><strong>Prostaglandin E2 Standard</strong></td>
<td><strong>Prostaglandin E2 Standard</strong></td>
</tr>
<tr>
<td>Reconstitute the PGE2 Standard with distilled water in order to obtain a 5000 pg/mL stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. This stock solution is stable 7 days at 4°C. It can be frozen and stored at -60°C or below and thawed once only.</td>
<td>Reconstitute the PGE2 Standard with distilled water in order to obtain a 5000 pg/mL stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. This stock solution is stable 7 days at 4°C. It can be frozen and stored at -60°C or below and thawed once only.</td>
</tr>
</tbody>
</table>

## TO PREPARE WORKING ANTIBODY SOLUTIONS:

Each well requires 5 µL of Anti-Prostaglandin E2-Eu<sup>3+</sup> Cryptate Antibody and 5 µL of Prostaglandin E2-d2. Prepare into separate vials the two antibody solutions.

<table>
<thead>
<tr>
<th>500 TESTS KIT - 62P2APEG</th>
<th>10,000 TESTS KIT - 62P2APEH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-Prostaglandin E2- Cryptate antibody</strong></td>
<td><strong>Anti-Prostaglandin E2-Cryptate antibody</strong></td>
</tr>
<tr>
<td>After reconstitution, the Anti-Prostaglandin E2-Cryptate antibody is ready to use.</td>
<td>Dilute 20-fold the stock solution of anti-Prostaglandin E2-cryptate antibody with detection buffer #3 e.g. take 1 mL of cryptate-antibody stock solution and add it to 19 mL of detection buffer #3.</td>
</tr>
<tr>
<td><strong>Prostaglandin E2-d2</strong></td>
<td><strong>Prostaglandin E2-d2</strong></td>
</tr>
<tr>
<td>After reconstitution, the Prostaglandin E2-d2 is ready to use.</td>
<td>Dilute 20-fold the stock solution of PGE2-d2 with detection buffer #3 e.g. take 1 mL of PGE2-d2 stock solution and add it to 19 mL of detection buffer #3.</td>
</tr>
<tr>
<td><strong>Antibody mix</strong></td>
<td><strong>Antibody mix</strong></td>
</tr>
<tr>
<td>Do not pre-mix the d2 and Cryptate solutions prior to dispensing.</td>
<td>Do not pre-mix the d2 and Cryptate solutions prior to dispensing.</td>
</tr>
</tbody>
</table>
**TO PREPARE WORKING STANDARD SOLUTIONS:**

- Each well requires 10 µL of standard.
- Dilute the standard stock solution serially with diluent.
- In order to check for a potential interference effect from your assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in diluent.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

Take 200 µL of the standard stock solution in order to prepare high standard (Std 7).

Use the high standard (Std 7) to prepare the standard curve using 1/3 serial dilutions as follows:

- Dispense 200µl of diluent into each vial from Std 6 to Std 0.
- Add 100µl of standard to 200µl of diluent, mix gently and repeat the 1/3 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Positive control) is diluent or appropriate culture medium alone.

<table>
<thead>
<tr>
<th>STANDARD</th>
<th>SERIAL DILUTIONS</th>
<th>PROSTAGLANDIN E2 WORKING SOLUTION (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Stock solution</td>
<td>Reconstituted lyophilisate</td>
<td>5,000</td>
</tr>
<tr>
<td>Standard 7</td>
<td>200µl standard stock solution</td>
<td>5,000</td>
</tr>
<tr>
<td>Standard 6</td>
<td>100 µL standard 7 + 200 µL Diluent</td>
<td>1,666.7</td>
</tr>
<tr>
<td>Standard 5</td>
<td>100 µL standard 6 + 200 µL Diluent</td>
<td>555.5</td>
</tr>
<tr>
<td>Standard 4</td>
<td>100 µL standard 5 + 200 µL Diluent</td>
<td>185.2</td>
</tr>
<tr>
<td>Standard 3</td>
<td>100 µL standard 4 + 200 µL Diluent</td>
<td>61.7</td>
</tr>
<tr>
<td>Standard 2</td>
<td>100 µL standard 3 + 200 µL Diluent</td>
<td>20.6</td>
</tr>
<tr>
<td>Standard 1</td>
<td>100 µL standard 2 + 200 µL Diluent</td>
<td>6.85</td>
</tr>
<tr>
<td>Standard 0</td>
<td>300µL Diluent</td>
<td>0</td>
</tr>
</tbody>
</table>

* Standard curve can be prepared in other appropriate medium
**TO PREPARE SAMPLES:**
- Each well requires 10 µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- All samples with a concentration above the highest standard (Std 7) must be diluted in diluent or in your appropriate sample medium.

### ASSAY PROTOCOL

<table>
<thead>
<tr>
<th>Step</th>
<th>Negative control (or Cryptate control)</th>
<th>Standard (Std 0 - Std 7)</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dispense 10 µL of diluent into each diluent well.</td>
<td>Dispense 10 µL of each Prostaglandin E2 standard (Std 0 - Std 7) into each standard well.</td>
<td>Dispense 10 µL of sample into each sample well.</td>
</tr>
<tr>
<td>2</td>
<td>Add 5 µL of detection buffer to all wells</td>
<td>Add 5 µL of Prostaglandin E2-d2 working solution to all wells</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Add 5 µL of Anti-Prostaglandin E2-Eu³⁺ Cryptate working solution to all wells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Seal the plate and incubate 5 hours @ RT or overnight at 4°C in order to improve the assay sensitivity.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Remove the plate sealer and read on an HTRF® compatible reader</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### A
- 10 µL Diluent (Negative control)
- 5 µL detection buffer
- 5 µL Anti-Prostaglandin E2-Eu³⁺ Cryptate

### B
- 10 µL Std 0 (Positive control)
- 5 µL Prostaglandin E2-d2
- 5 µL Anti-Prostaglandin E2-Eu³⁺ Cryptate

### C
- 10 µL Std 1
- 5 µL Prostaglandin E2-d2
- 5 µL Anti-Prostaglandin E2-Eu³⁺ Cryptate

### D
- 10 µL Std ...
- 5 µL Prostaglandin E2-d2
- 5 µL Anti-Prostaglandin E2-Eu³⁺ Cryptate

### E
- 10 µL Std ...
- 5 µL Prostaglandin E2-d2
- 5 µL Anti-Prostaglandin E2-Eu³⁺ Cryptate

### F
- 10 µL Std ...
- 5 µL Prostaglandin E2-d2
- 5 µL Anti-Prostaglandin E2-Eu³⁺ Cryptate

### G
- 10 µL Std ...
- 5 µL Anti-Prostaglandin E2-d2
- 5 µL Prostaglandin E2-Eu³⁺ Cryptate

### H
- 10 µL Std ...
- 5 µL Prostaglandin E2-d2
- 5 µL Anti-Prostaglandin E2-Eu³⁺ Cryptate

### I
- 10 µL Std ...
- 5 µL Prostaglandin E2-d2
- 5 µL Anti-Prostaglandin E2-Eu³⁺ Cryptate

### J
- 10 µL Sample 1
- 5 µL Prostaglandin E2-d2
- 5 µL Anti-Prostaglandin E2-Eu³⁺ Cryptate

### K
- Repeat Well A1
- Repeat Well A1

### L
- Repeat Well B1
- Repeat Well B1

### M
- Repeat Well C1
- Repeat Well C1

### N
- Repeat Well D1
- Repeat Well D1

### O
- Repeat Well E1
- Repeat Well E1

### P
- Repeat Well F1
- Repeat Well F1

### Q
- Repeat Well G1
- Repeat Well G1

### R
- Repeat Well H1
- Repeat Well H1

### S
- Repeat Well I1
- Repeat Well I1

### T
- Repeat Well A4
- Repeat Well A4

### U
- Repeat Well B4
- Repeat Well B4

### V
- Repeat Well C4
- Repeat Well C4

### W
- Repeat Well D4
- Repeat Well D4

### X
- Repeat Well E4
- Repeat Well E4

### Y
- Repeat Well F4
- Repeat Well F4

### Z
- Repeat Well G4
- Repeat Well G4

### AA
- Repeat Well H4
- Repeat Well H4

### AB
- Repeat Well I4
- Repeat Well I4
DATA REDUCTION & INTERPRETATION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

\[
\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4
\]

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

\[
\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100
\]

3. Calculate the % delta F which reflects the signal to background of the assay. The negative control plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

\[
\text{delta F (\%)} = \frac{\text{Ratio Standard or sample} - \text{Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100
\]

For more information about data reduction, please visit http://www.cisbio.com/htrf-ratio-and-data-reduction

RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example. Results may vary from one HTRF® compatible reader to another.

The assay standard curve is created by plotting delta F% versus the analyte concentration.

<table>
<thead>
<tr>
<th></th>
<th>Ratio (1)</th>
<th>CV (2)</th>
<th>Delta F% (3) (5H RT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>410</td>
<td>0.1%</td>
<td></td>
</tr>
<tr>
<td>Std 0 – Positive control</td>
<td>15,855</td>
<td>1.2%</td>
<td>3.767%</td>
</tr>
<tr>
<td>Std 1 - 6.8 pg/mL</td>
<td>15,486</td>
<td>0.7%</td>
<td>3.677%</td>
</tr>
<tr>
<td>Std 2 - 20.6 pg/mL</td>
<td>14,748</td>
<td>2.5%</td>
<td>3.497%</td>
</tr>
<tr>
<td>Std 3 - 61.7 pg/mL</td>
<td>12,826</td>
<td>0.7%</td>
<td>3.028%</td>
</tr>
<tr>
<td>Std 4 - 185.2 pg/mL</td>
<td>9,442</td>
<td>1.9%</td>
<td>2.203%</td>
</tr>
<tr>
<td>Std 5 - 555.5 pg/mL</td>
<td>5,208</td>
<td>0.7%</td>
<td>1.170%</td>
</tr>
<tr>
<td>Std 6 - 1,666.7 pg/mL</td>
<td>2,391</td>
<td>0.3%</td>
<td>483%</td>
</tr>
<tr>
<td>Std 7 - 5,000 pg/mL</td>
<td>1,138</td>
<td>0.9%</td>
<td>178%</td>
</tr>
</tbody>
</table>

![PGE2 - Standard curve](image)
ANALYTICAL CHARACTERISTICS

DETECTION LIMIT & EC50 WORKING CONCENTRATIONS

<table>
<thead>
<tr>
<th></th>
<th>Detection limit</th>
<th>EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>incubation 5 hours at RT</td>
<td>&lt; 20 pg/mL (&lt;57 pM)</td>
<td>250 pg/mL (0.7 nM)</td>
</tr>
</tbody>
</table>

CROSS-REACTIVITY

<table>
<thead>
<tr>
<th></th>
<th>Cross-reactivity in %</th>
<th></th>
<th>Cross-reactivity in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8-iso prostaglandin F2 alpha</td>
<td>0.46</td>
</tr>
<tr>
<td>Prostaglandin E1</td>
<td>78.4</td>
<td>PGF2 alpha</td>
<td>2</td>
</tr>
<tr>
<td>Prostaglandin E3</td>
<td>32.4</td>
<td>arachidonic acid</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Sulprostone</td>
<td>11.4</td>
<td>Prostaglandin A1</td>
<td>0.08</td>
</tr>
<tr>
<td>6-keto prostaglandin F1 alpha</td>
<td>1.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostaglandin B1</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostaglandin B2</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostaglandin D2</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thromboxane B2</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>