MANUAL

HTRF PROSTAGLANDIN E2 DETECTION KITS

Part # 62P2APEG & 62P2APEH

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

Revision: #07 of September 2024

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

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

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Native Prostaglandin E2 produced by cells in buffered solution or in cell culture supernatants and offers a fast alternative to ELISA.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, Prostaglandin E2 is detected in a competitive assay by using anti Prostaglandin E2 antibody labeled with Europium cryptate (donor), and Prostaglandin E2 labeled with d2 (acceptor).

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 HTRF detection solutions and thereby prevents FRET from occurring. The specific signal is inversely proportional to the Prostaglandin E2 concentration.

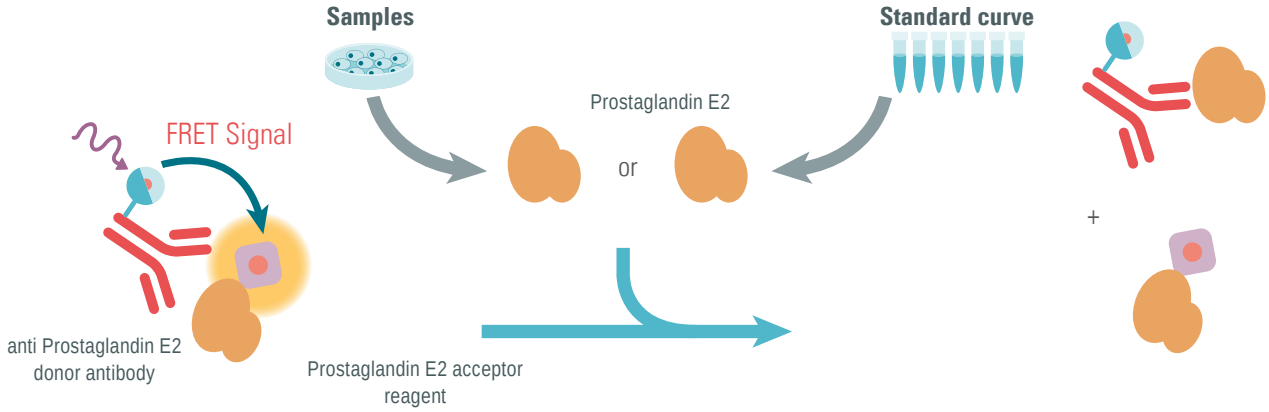
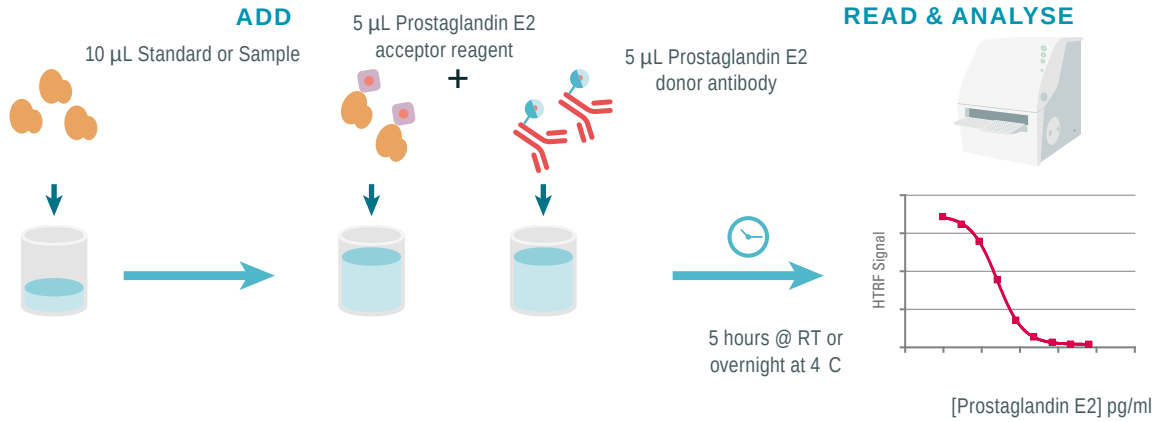


Figure 1: Principle of HTRF Prostaglandin E2 competitive assay.

MANUAL AT A GLANCE



Do not pre-mix the d2 and Cryptate solutions prior to dispensing Make sure to use the set-up for Eu Cryptate.

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 62P2APEG	10,000 TESTS * CAT # 62P2APEH
Prostaglandin E2 Standard Lyophilized	1 vial Concentrated PGE2	1 vial Concentrated PGE2
anti Prostaglandin E2 antibody Eu Cryptate antibody	1 vial Lyophilized	1 vial Lyophilized
Prostaglandin E2 d2 reagent	1 vial Lyophilized	1 vial Lyophilized
Diluent ** ready-to-use	1 vial 20 mL	1 vial 20 mL
Detection buffer *** ready to use	1 vial 7 mL	1 vial 105 mL

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

PURCHASE SEPARATELY:

- HTRF®-Certified Reader. Make sure the setup for Eu Cryptate is used.

For a list of HTRF-compatible readers and set-up recommendations, please visit www.revvity.com

- Small volume (SV) detection microplates - Use white plate only..

For more information about microplate recommendations, please visit our website at: www.revvity.com

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, reconstituted reagents, antibodies, and standard stock solutions may be frozen and 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 (Can be stored 7 days at 4°C) .

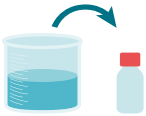
REAGENT PREPARATION

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 Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- 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.

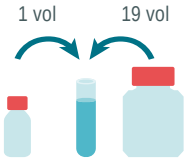
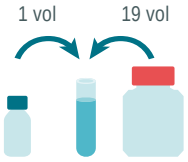
TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 62P2APEG		10,000 TESTS KIT - 62P2APEH	
anti Prostaglandin E2 antibody Eu Cryptate antibody			
Reconstitute the anti Prostaglandin E2 antibody Eu Cryptate antibody with 2.5 mL detection buffer. Mix gently. This ready to use 1X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.			Reconstitute the anti Prostaglandin E2 antibody Eu Cryptate antibody with 2.5 mL distilled water. Mix gently. This 20X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.
Prostaglandin E2 d2 reagent			
Reconstitute the Prostaglandin E2 d2 reagent with 2.5 mL detection buffer. Mix gently. This ready to use 1X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.			Reconstitute the Prostaglandin E2 d2 reagent with 2.5 mL distilled water. Mix gently. This 20X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.
Prostaglandin E2 Standard			
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.			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.
Diluent			
The diluent is ready-to-use			The diluent is ready-to-use
Detection buffer			
The Detection buffer is ready-to-use.			The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 5 µL anti Prostaglandin E2 antibody Eu Cryptate antibody and 5 µL Prostaglandin E2 d2 reagent.

Prepare the two solutions in separate vials.

500 TESTS KIT - 62P2APEG		10,000 TESTS KIT - 62P2APEH	
anti Prostaglandin E2 antibody Eu Cryptate antibody			
After reconstitution, the Prostaglandin Eu Cryptate antibody is ready to use.			Dilute 20-fold the stock solution of Prostaglandin E2 Eu Cryptate antibody with detection buffer#3 e.g. take 1 mL of Eu Cryptate antibody stock solution and add it to 19 mL of detection buffer #3.
Prostaglandin E2 d2 reagent			
After reconstitution, the Prostaglandin d2 reagent is ready to use.			Dilute 20-fold the stock solution of Prostaglandin E2 d2 reagent with detection buffer#3 e.g. take 1 mL of d2 reagent stock solution and add it to 19 mL of detection buffer #3.
Antibody mix			
Do not pre-mix the d2 and the Eu Cryptate solutions prior to dispensing.			

TO PREPARE STANDARD WORKING 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 own 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:

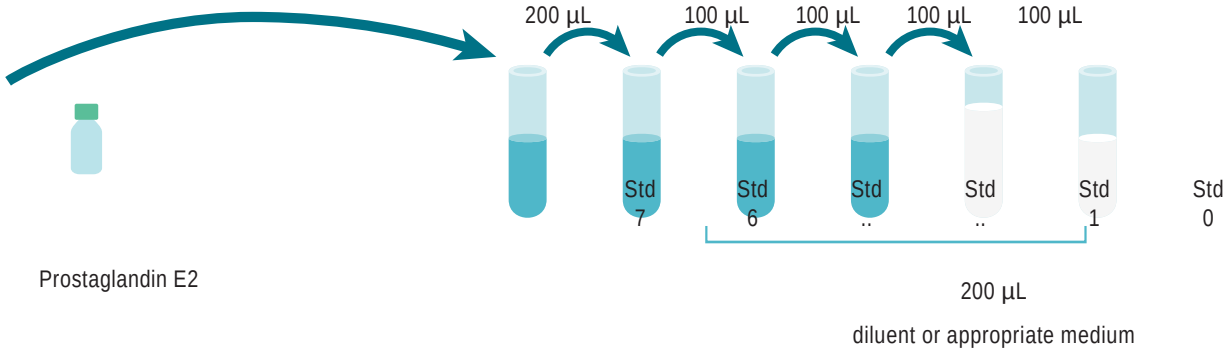
Reconstitute the PGE2 standard according to the instructions on the vial label. This leads to high standard (Std 7)

Dilute the with diluent to prepare high standard (Std 7): e.g. take 200 µL of and add it to 200 µL of diluent . Mix gently.

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

- Dispense 200 µL of diluent in each vial from Std 6 to Std 0.
- Add 100 µL of standard to 200 µL 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.



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

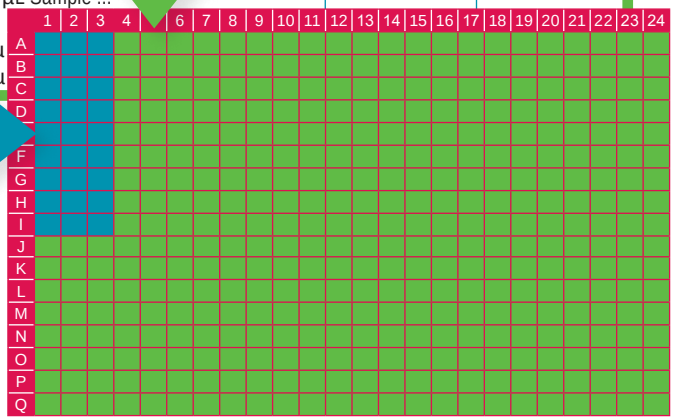
TO PREPARE SAMPLES:

- 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.
- Samples with a concentration above the highest standard (Std 7) must be diluted diluent or in your appropriate sample medium.

ASSAY MANUAL

	Negative control or Cryptate control	Standard (Std 0 - Std 7)	Samples
Step 1 	Dispense 10 µL of diluent into each negative control well	Dispense 10 µL of each Prostaglandin E2 standard (Std 0 - Std 7) into each standard well	Dispense 10 µL of each sample into each sample well
Step 2 	Add 5 µL of Detection buffer to all negative control wells	Add 5 µL Prostaglandin E2 acceptor reagent working solution to all wells	
Step 3 	Add 5 µL Prostaglandin E2 donor antibody working solution to all wells		
Step 4 	Seal the plate and incubate 5 hours @ RT or overnight at 4°C In order to improve the assay sensitivity.		
Step 5	Remove the plate sealer and read on an HTRF® compatible reader		

	1	2	3	4	5	6
A	10 µL diluent (Negative control) 5 µL 5 µL Prostaglandin E2 donor antibody	Repeat Well A1	Repeat Well A1	10 µL Sample 1 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well A4	Repeat Well A4
B	10 µL Std 0 (Positive control) 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well B1	Repeat Well B1	10 µL Sample 2 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well B4	Repeat Well B4
C	10 µL Std 1 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well C1	Repeat Well C1	10 µL Sample 3 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well C4	Repeat Well C4
D	10 µL Std 2 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well D1	Repeat Well D1	10 µL Sample ... 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well D4	Repeat Well D4
E	10 µLStd ... 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well E1	Repeat Well E1	10 µL Sample ... 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well E4	Repeat Well E4
F	10 µL Std ... 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well F1	Repeat Well F1	10 µL Sample ... 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well F4	Repeat Well F4
G	10 µL Std ... 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well G1	Repeat Well G1	10 µL Sample ... 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostagla...r antibody	Repeat Well G4	Repeat Well G4
H	10 µL Std ... 5 µL Prostaglandin E2 acceptor reagent 5 µL Prostaglandin E2 donor antibody	Repeat Well H1	Repeat Well H1	10 µL Sample ... 5 µ 5 µ		



DATA REDUCTION

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.

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

For more information about data reduction, please visit www.revvity.com

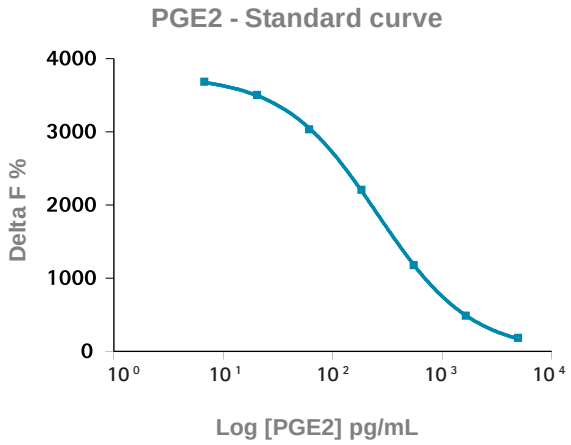
RESULTS

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.

	Ratio (1)	CV (2)	Delta F% (3) (5H RT)
Negative control	410	0.1%	
Std 0 – Positive control	15,855	1.2%	3,767%
Std 1 - 6.8 pg/mL	15,486	0.7%	3,677%
Std 2 - 20.6 pg/mL	14,748	2.5%	3,497%
Std 3 - 61.7 pg/mL	12,826	0.7%	3,028%
Std 4 - 185.2 pg/mL	9,442	1.9%	2,203%
Std 5 - 555.5 pg/mL	5,208	0.7%	1,170%
Std 6 - 1,666.7 pg/mL	2,391	0.3%	483%
Std 7 - 5,000 pg/mL	1,138	0.9%	178%



ANALYTICAL CHARACTERISTICS

DETECTION LIMIT & EC50 WORKING CONCENTRATIONS

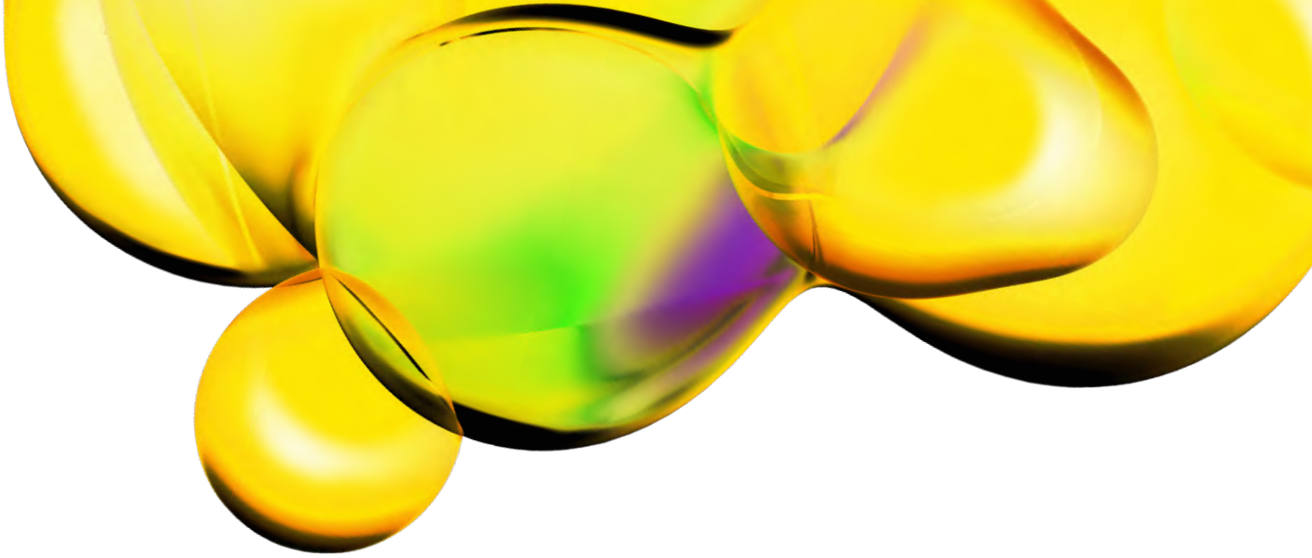
	Detection limit	EC50
incubation 5 hours at RT	< 20 pg/mL (<57 pM)	250 pg/mL (0.7 nM)

CROSS-REACTIVITY

	Cross-reactivity in %
Prostaglandin E1	78.4
Prostaglandin E3	32.4
Sulprostone	11.4
6-keto prostaglandin F1 alpha	1.22

	Cross-reactivity in %
8-iso prostaglandin F2 alpha	0.46
PGF2 alpha	2
arachidonic acid	< 0.01
Prostaglandin A1	0.06

	Cross-reactivity in %
Prostaglandin B1	<0.01
Prostaglandin B2	<0.01
Prostaglandin D2	<0.01
Thromboxane B2	<0.01



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