Version 22-09/25

User's Manual

Prostaglandin E2 ELISA Kit

REF **DEIA4977**

Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

PGE2 ELISA Kit is a competitive assay that can be used for quantification of PGE2 in urine, plasma, tissue culture supernatants, and other sample matrices. The assay has a range from 7.8-1,000 pg/ml, a sensitivity (80% B/B0) of approximately 13 pg/ml, and a lower limit of detection (LLOD) of 11 pg/ml.

General Description

Prostaglandin E2 (PGE2) is an eicosanoid formed from arachidonic acid by COX enzymes and PGE2 synthase (PGES) via PGG2 and PGH2 intermediates. It can be produced de novo in all cells following cell activation and the release of arachidonic acid from plasma membrane phospholipids or when exogenous free arachidonate is available. It acts in an autocrine or paracrine fashion to bind to its receptors, EP1, EP2, EP3, and EP4, which have differing tissue distribution and cellular localizations, to initiate signaling through various pathways, including the protein kinase A (PKA), β-catenin, EGF, MAPK, NF-κB, and PI3K/Akt pathways. Due to this, PGE2 is involved in a wide variety of biological processes, including inflammation, fertility and parturition, gastric mucosal integrity and motility, and immune modulation in and outside of cancer.

Principles of Testing

This assay is based on the competition between PGE2 and a PGE2-acetylcholinesterase (AChE) conjugate (PGE2 Tracer) for a limited amount of PGE2 Monoclonal Antibody. Because the concentration of the PGE2 Tracer is held constant while the concentration of PGE2 varies, the amount of PGE2 Tracer that is able to bind to the PGE2 Monoclonal Antibody will be inversely proportional to the concentration of PGE2 in the well. This antibody-PGE2 complex binds to goat polyclonal anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PGE2 Tracer bound to the well, which is inversely proportional to the amount of free PGE2 present in the well during the incubation; or

Absorbance ∝ [Bound PGE2 Tracer] ∝ 1/[PGE2]

Reagents And Materials Provided

1. Prostaglandin E2 Monoclonal Antibody, 1 vial/100 dtn (96 wells), 1 vial/500 dtn(480 wells)
2. Prostaglandin E2 AChE Tracer, 1 vial/100 dtn (96 wells), 1 vial/500 dtn(480 wells)
3. Prostaglandin E2 ELISA Standard, 1 vial (96 wells), 1 vial(480 wells)
4. ELISA Buffer Concentrate (10×), 2 vials/10 ml (96 wells), 4 vials/10 ml(480 wells)
5. Wash Buffer Concentrate (400×), 1 vial/5 ml (96 wells), 1 vial/12.5 ml(480 wells)
6. Polysorbate 20, 1 vial/3 ml (96 wells), 1 vial/3 ml(480 wells)
7. Goat Anti-Mouse IgG Coated Plate, 1 plate (96 wells), 5 plates(480 wells)
8. 96-Well Cover Sheet, 1 cover (96 wells), 5 covers(480 wells)

9. Ellman' s Reagent, 3 vials/100 dtn (96 wells), 6 vials/250 dtn(480 wells)
10. ELISA Tracer Dye, 1 vial (96 wells), 1 vial(480 wells)
11. ELISA Antiserum Dye, 1 vial (96 wells), 1 vial(480 wells)

Materials Required But Not Supplied

1. A plate reader capable of measuring absorbance between 405-420 nm.
2. Adjustable pipettes; multichannel or repeating pipettor recommended.
3. A source of 'UltraPure' water. Water used to prepare all ELISA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for ELISA.
4. Materials used for Sample Preparation

Storage

This kit will perform as specified if stored as directed at -20°C and used beforethe expiration date indicated on the outside of the box.

Specimen Collection And Preparation

This assay has been validated for a wide range of samples including urine, plasma, and tissue culture media. Proper sample storage and preparation are essential for consistent and accurate results. Please read this section thoroughly before beginning the assay.

General Precautions

1. All samples must be free of organic solvents prior to assay.
2. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.3. Samples of mouse and rat origin may contain antibodies which interfere with the assay by binding to the goat anti-mouse plate. We recommend that all mouse and rat samples be purified prior to use in this assay.

Urine

Since interference in urine is infrequent,dilutions of 1:2and greater show a directlinear correlation between PGE2 immunoreactivity and PGE2 concentration. However,the amount of PGE2 in normal urine is very low in comparison withother potentially immunoreactive metabolites.

Plasma

Collect blood in vacutainers containing heparin,EDTA,or sodium citrate.Indomethacin should be added immediately after whole blood collection(sufficient to give a 10μM final concentration). Indomethacin will prevent exvivo formation of eicosanoids,which have the potential to interfere with thisassay (although most eicosanoids do not appear to exhibit any cross reactivity).

The amount of PGE2 in normal plasma is very low in comparison with other potentially immunoreactive metabolites. In addition, plasma is a complex matrix that contains many substances that can interfere with this assay and thereforesample purification is recommended. By purifying a large volume of sample(5-10

ml),the PGE2 content can be concentrated into as little as 0.5 ml of ELISA Buffer. This will bring the PGE2 concentration into the readable range of the standard curve.

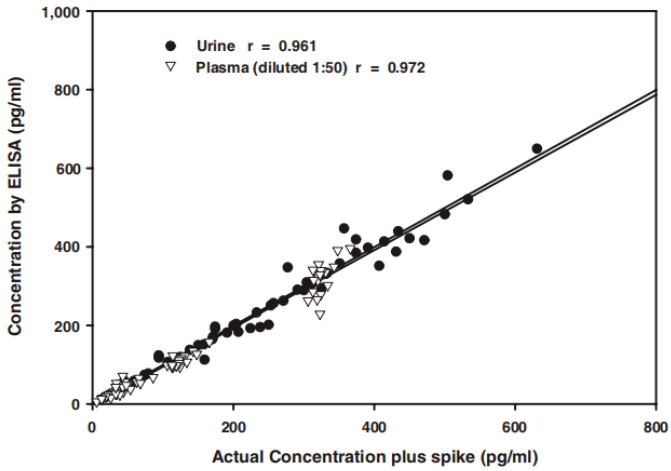


Figure 2. Validation curves for this PGE_2 assay

Culture Medium Samples

Cell culture supernatants may be assayed directly without purification.If the PGE2 concentration in the medium is high enough to dilute the sample 10-fold with ELISA Buffer,the assay can be performed without any modifications.When assaying less concentrated samples(where samples cannot be diluted with ELISA Buffer),dilute the standard curve in the same culture medium as that used in the experiment. This will ensure that the matrix for the standards is comparable tothe samples.We recommend that a standard curve be run first to ensure that theassay will perform in a particular culture medium.

Tissue Samples

Snap-freeze tissues in liquid nitrogen immediately upon collection and then store at -80°C. Add 5 ml homogenization buffer (0.1 M phosphate, pH 7.4, containing 1 mM EDTA and 10 µM indomethacin) to 1 g of tissue. Homogenize the sample with either a Polytron-type homogenizer or a sonicator. Proceed to the purification section below. Alternatively, frozen samples can be pulverized in the presence of ethanol for extraction of PGE2.

Tissue Homogenization using the Precellys 24 Homogenizer

Snap-freeze tissues in liquid nitrogen immediately upon collection and store at -80°C. Add 1 ml homogenization buffer (0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA and 10 µM indomethacin) per 100 mg of tissue. Homogenize the sample with the Precellys 24 using the appropriate settings (see Table 1). Spin the tissue homogenates at 8,000 x g for 10 minutes. Collect supernatant and assay as described below. Samples will need to be diluted appropriately for the assay. Tissue samples should be normalized using a protein assay.

Organ	Speed (rpm)	Cycle Length (seconds)	Beads
Lung	5,200	20	CK28 Large Ceramic
Brain	5,500	20	CK28 Large Ceramic
Liver	5,200	15	CK28 Large Ceramic
Kidney	5,200	20	CK14 Small Ceramic
Heart	5,200	30	CK14 Small Ceramic

Testing for Interference

Plasma, serum, as well as other heterogeneous mixtures such as CSF often contain contaminants which can interfere in the assay. It is best to check for interference to evaluate the need for sample purification before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample between 10 and 250 pg/ml (i.e., between 20-80% B/B0). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated PGE2 concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised.

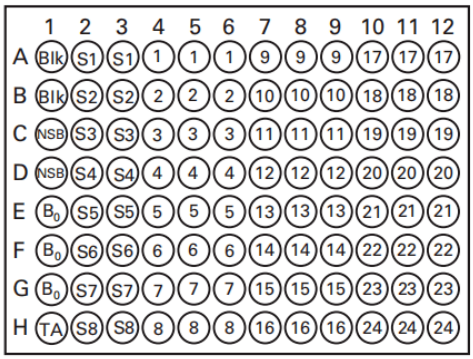
Plate Preparation

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. **NOTE:** If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.

Each plate or set of strips must contain a minimum of two blanks (Blk), two non-specific binding wells (NSB), two maximum binding wells (B0), and an eight point standard curve run in duplicate. **NOTE:** Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure, below. The user may vary the location and type of wells present as necessary for each particular experiment.



Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
B_0 - Maximum Binding
S1-S8 - Standards 1-8
1-24 - Samples

Reagent Preparation

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for approximately two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. ELISA Buffer Preparation

Dilute the contents of one vial of ELISA Buffer Concentrate (10×) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. **NOTE:** It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.

2. Wash Buffer Preparation

5 ml vial Wash Buffer Concentrate (400×) (96-well kit): Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20.

OR

12. 5 ml vial Wash Buffer Concentrate (400×) (480-well kit): Dilute to a total volume of 5 liters with UltraPure water and add 2.5 ml of Polysorbate 20.

Smaller volumes of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Polysorbate 20 (0.5 ml/liter of Wash Buffer).

NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

SPE (C-18) Purification Protocol

The following protocol is a suggestion only. You may choose a different protocol based on your own requirements, sample type, and expertise.

Materials Needed:

1. 1 M acetate buffer, deionized water, ethanol, methanol, and ethyl acetate
2. 500 mg SPE Cartridges (C-18)

SPE (C18) Purification Protocol:

1. Aliquot a known amount of each sample into a clean test tube (500 µl is recommended). If your samples need to be concentrated, a larger volume should be used (e.g., a 5 ml sample will be concentrated by a factor of 10, a 10 ml sample will be concentrated by a factor of 20, etc.).
2. Precipitation of proteins using ethanol is optional and may not be needed if samples are clean enough to flow through the SPE Cartridge (C-18). Body fluids such as plasma and urine can typically be applied directly to the SPE Cartridge (C-18) after the acidification step (step 4) below. To precipitate proteins, add ethanol (approximately four times the sample volume) to each tube. Vortex to mix thoroughly. Incubate samples at 4°C for five minutes, then centrifuge at 3,000 x g for 10 minutes to remove precipitated proteins. Transfer the supernatant to a clean test tube. Evaporate the ethanol under nitrogen.
3. Acidify the sample to ~pH 4.0 by the addition of 1 M acetate buffer (or citrate buffer). (Standardize the pH adjustment using the sample matrix prior to proceeding with a large number of samples). If the samples are cloudy or contain precipitate, either filter or centrifuge to remove the precipitate. Particulate matter in the sample may clog the SPE Cartridge (C-18).
4. Prepare SPE (C-18) columns by rinsing with 5 ml methanol followed by 5 ml deionized water. Do not allow the SPE Cartridge (C-18) to dry.
5. Apply the sample to the SPE Cartridge (C-18) and allow the sample to completely enter the packing

material.

6. Wash the column with 5 ml deionized water. Discard the wash.
7. Elute the PGE2 from the column with 5 ml ethyl acetate containing 1% methanol. Higher recovery and better reproducibility may be obtained if the sample is applied and eluted by gravity. The wash steps may be performed under vacuum or pressure.
8. Evaporate the ethyl acetate to dryness under a stream of nitrogen. It is very important that all of the organic solvent be removed as even small quantities will adversely affect the ELISA.
9. To resuspend the sample, add 500 µl ELISA Buffer. Vortex. It is common for insoluble precipitate to remain in the sample after addition of ELISA Buffer; this will not affect the assay. This sample is now ready for use in the ELISA.
10. Use 50 µl of the resuspended sample for scintillation counting.

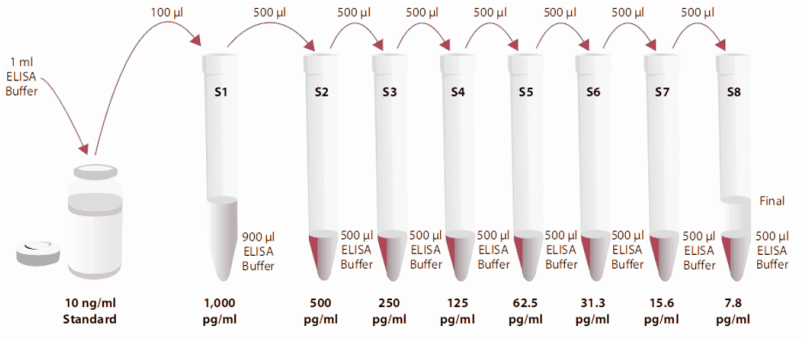
Preparation of Assay-Specific Reagents

1. Prostaglandin E2 ELISA Standard

Reconstitute the contents of the PGE2 ELISA Standard with 1.0 ml of ELISA Buffer. The concentration of this solution (the bulk standard) will be 10 ng/ml. Stored at 4°C; this standard will be stable for up to 4 weeks.

NOTE: If assaying culture medium samples that have not been diluted with ELISA Buffer, culture medium should be used in place of ELISA Buffer for dilution of the standard curve.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl ELISA Buffer to tube #1 and 500 µl ELISA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (10 ng/ml) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, will be 1 ng/ml (1,000 pg/ml). Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than 24 hours.



2. Prostaglandin E2 AChE Tracer

Reconstitute the PGE2 AChE Tracer as follows:

100 dtn PGE2 AChE Tracer (96-well kit): Reconstitute with 6 ml ELISA Buffer.

OR

500 dtn PGE2 AChE Tracer (480-well kit): Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted PGE2 AChE Tracer at 4°C (do not freeze!) and use within four weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer or add 300 µl of dye to 30 ml of tracer).

3. Prostaglandin E2 Monoclonal Antibody

Reconstitute the PGE2 Monoclonal Antibody as follows:

100 dtn PGE2 Monoclonal Antibody (96-well kit): Reconstitute with 6 ml ELISA Buffer.

OR

500 dtn PGE2 Monoclonal Antibody (480-well kit): Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted PGE2 Monoclonal Antibody at 4°C. It will be stable for at least four weeks. A 20% surplus of antibody has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antibody, if desired, to aid in visualization of antibody-containing wells. Add the dye to the reconstituted antibody at a final dilution of 1:100 (add 60 µl of dye to 6 ml antibody or add 300 µl of dye to 30 ml of antibody).

Assay Procedure

Pipetting Hints

1. Use different tips to pipette each reagent.
2. Before pipetting each reagent,equilibrate the pipette tip in that
3. Do not expose the pipette tip to the reagent(s)already in the well. reagent (i.e.,slowly fill the tip and gently expel the contents,repeatseveral times).

Addition of the Reagents

1. ELISA Buffer

Add 100 µl ELISA Buffer to NSB wells.Add 50µl ELISA Buffer to $B_o$wells.If culture medium was used to dilute the standard curve, substitute 50µlof culture medium for ELISA Buffer in the NSB and $B_o$wells (i.e.,add 50µl culture medium to NSB and B_0 wells and 50 µl ELISA Buffer to NSB wells).

2. Prostaglandin E_2ELISA Standard

Add 50µl from tube #8 to both of the lowest standard wells(S8). Add 50µl from tube#7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip shouldbe used to aliquot all the standards. Before pipetting each standard, be sureto equilibrate the pipette tip in that standard.

3. Samples

Add 50 µl of sample per well. Each sample should be assayed at a minimumof two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. Prostaglandin E2 AChE Tracer

Add 50µl to each well except the TA and the Blk wells.

5. Prostaglandin E2 Monoclonal Antibody

Add 50 µl to each well except the TA,the NSB,and the Blk wells.

Well	ELISA Buffer	Standard/Sample	Tracer	Antibody
Blk	-	-	-	-
TA	-	-	5 µl (at devl. step)	-
NSB	100 µl	-	50 µl	-
B_0	50 µl	-	50 µl	50 µl
Std/Sample	-	50 µl	50 µl	50 µl

6. Incubation of the Plate

Cover each plate with plastic film and incubate 18 hours at 4°C.

7. Development of the Plate

a. Reconstitute Ellman's Reagent immediately before use(20 ml of reagent issufficient to develop 100 wells):

100 dtn vial Ellman's Reagent(96-well kit;Item No.400050):Reconstitutewith 20 ml of UltraPure water.

OR

250 dtn vial Ellman's Reagent(480-well kit;Item No.400050):Reconstitutewith 50 ml of UltraPure water.

NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day itis prepared; protect the Ellman's Reagent from light when not in use. Extra vials of there agent have been provided should a plate need to be re-developed or multiple assays run on different days.

b. Empty the wells and rinse five times with Wash Buffer.

c. Add 200µl of Ellman's Reagent to each well.

d. Add 5µl of tracer to the TA wells.

e. Cover the plate with plastic film. Optimum development is obtained byusing an orbital shaker equipped with a large,flat cover to allow the plate(s)to develop in the dark. This assay typically develops (i.e.,$B_o$wells ≥0.3 A.U.(blank subtracted))in 60-90 minutes.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. **NOTE:** Any loss of Ellman's Reagent will affect theabsorbance readings. If Ellman's Reagent is present on the cover, use a pipetteto transfer the Ellman's Reagent into the well. If too much Ellman's Reagent has splashed on the cover to easily redistribute back into the wells, wash the plate three times with wash buffer and repeat the development with fresh Ellman's Reagent.

Calculation

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively a

spreadsheet program can be used. The data should be plotted as either %B/B_0 versus log concentration using a four-parameter logistic fit oras logit B/B_0 versus log concentration using a linear fit.

Calculations

Preparation of the Data:

The following procedure is recommended for preparation of the data prior tographical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blankwells from the absorbance readings of the rest of the plate,be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average.This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for theremaining wells.To do this,subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3).Repeat for S2-S8 and all sample wells.(To obtain %B/B_0 for a logistic four-parameterfit,multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather,theyare used as a diagnostic tool. Low or no absorbance from a TA well could indicate adysfunction in the enzyme-substrate system.

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 versus PGE2 concentration using linear(y)and log(x)axes and perform a 4-parameter logistic fit.

Alternative Plot -The data can also be lineraized using a logit transformation.The equation for this conversion is shown below. **NOTE:** Do not use %B/B_0 in this calculation.

logit(B/B_0)=ln [B/B_0/(1-B/B_0)]

Plot the data as logit(B/B_0)versus log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B_0 (or %B/B_0)value foreach sample. Determine the concentrationof each sample using the equation obtained from the standard curve plot. **NOTE:** Remember to account for any concentration or dilution of the sample prior to theaddition to the well. Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of thestandard curve.A 20% or greater disparity between the apparent concentrationof two different dilutions of the same sample indicates interference which couldbe eliminated by purification.

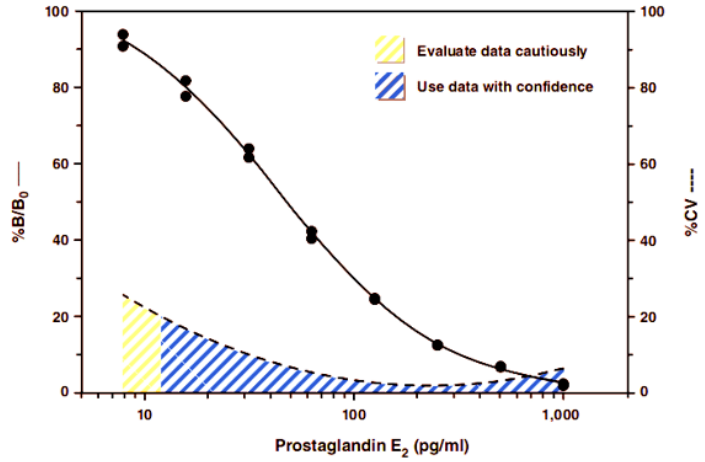
Typical Standard Curve

Sample Data

The standard curve presented here is an example of the data typically produced with this kit;however, your results will not be identical to these. You must run anew standard curve.Do not use the data below to determine the values of your samples. Your results could differ substantially.

	Raw Data		Average	Corrected
Total Activity	0.689	0.692	0.691	
NSB	0.004	0.002	0.003	
B_0	0.871	0.830		
	0.856	0.821	0.845	0.842

Dose (pg/ml)	Raw Data		Corrected		%B/B_0	
1,000	0.021	0.017	0.018	0.014	2.1	1.7
500	0.058	0.060	0.055	0.057	6.5	6.8
250	0.106	0.107	0.103	0.104	12.2	12.4
125	0.207	0.210	0.204	0.207	24.2	24.6
62.5	0.358	0.342	0.355	0.339	42.2	40.3
31.3	0.541	0.521	0.538	0.518	63.9	61.5
15.6	0.691	0.656	0.688	0.653	81.7	77.6
7.8	0.793	0.767	0.790	0.764	93.8	90.7



Assay Range = 7.8-1,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 13 pg/ml
Mid-point (defined as 50% B/B_0) = 30-70 pg/ml
Lower Limit of Detection (LLOD) = 11 pg/ml

The standard was diluted with ELISA Buffer.

Precision

The intra-and inter-assay CVs have been determined at multiple points on thestandard curve.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
1,000	4.2	12.4
500	3.9	6.4
250	3.8	7.8
125	3.7	11.6
62.5	6.6	15.5
31.3	7.8	15.0
15.6	10.1	20.9
7.8	30.4	35.0

Intra-and inter-assay variation

*%CV represents the variation in concentration(not absorbance)as determinedusing a reference standard curve.

Detection Range

7.8-1000 pg/ml

Detection Limit

Lower limit of detection (LLOD): 11 pg/ml

Sensitivity

Sensitivity (80% B/B_0): 13 pg/ml