Human Prostaglandin E_2 ELISA Kit

Catalog Number KHL1701 (96 tests)

Pub. No. MAN0014767 **Rev.** 3.0 (31)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human Prostaglandin E_2 ELISA Kit is a competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human prostaglandin E_2 in biological samples. The assay will recognize both natural and recombinant human prostaglandin E_2. This assay is based on the competition between Prostaglandin E_2 (PGE_2) and a PGE_2 alkaline phosphatase tracer for a limited amount of PGE_2 specific monoclonal antibody. Because of the competition between PGE_2 in the sample and PGE_2 tracer for the PGE_2 antibody, the signal obtained with the assay will be inversely proportional to the amount of PGE_2 in each sample.

PGE_2 is a potent lipid mediator produced by metabolism of arachidonic acid via the cyclooxygenase (COX) pathway. Following cellular stimulation, arachidonic acid is hydrolyzed from phospholipids stores by one of a family of phospholipase A_2 enzymes and converted to the unstable endoperoxide PGH_2 by either COX-1 or COX-2. PGH_2 is isomerized to PGE_2 by one of 3 distinct enzymes - microsomal PGES-1 (mPGES-1), mPGES-2, or a cytosolic enzyme, cPGES. PGE_2 is active in a number of physiological systems and settings including inflammation, immune regulation, generation of fever, pain perception, protection of the gastric muscosa, fertility and parturition, as well as sodium and water retention. The effects of PGE_2 are transduced by 4 subtypes of G protein-coupled receptors designated EP_1, EP_2, EP_3, EP_4, with K_d values for PGE_2 ranging from 1-10 nM. PGE_2 is rapidly metabolized in vivo by the prostaglandin 15-dehydrogenase pathway (15-hydroxy PGDH) to the inactive metabolite 13,14-dihydro-15-keto PGE_2. The half-life of PGE_2 in the circulatory system is ~30 seconds and normal plasma levels are 3-12 pg/mL.

Contents and storage

Upon receipt, store the kit at –20°C.

Contents	Cat. No. KHL1701
Prostaglandin E_2 Standard in ethanol	0.5 mL
Prostaglandin E_2 AP Tracer, lyophilized; contains 0.05% sodium azide	1 vial
Tris Buffer Concentrate; contains 0.5% sodium azide	2 × 10 mL
Antibody Coated Plate, 96-well plate	1 plate
Prostaglandin E_2 Monoclonal Antibody, lyophilized; contains 0.05% sodium azide	1 vial
Wash Buffer Concentrate	5 mL
DEA Buffer Concentrate	2.5 mL
pNPP Tablets	5 tablets
Plate Cover, adhesive strips	1

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes; beakers, flask, and cylinders for reagent preparation
- Orbital microplate shaker set to approximately 100 rpm
- 1.0 M HCl
- Methanol
- Hexane
- Ethyl acetate
- SPE C-18 Cartridges

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare Wash Buffer

Dilute the 5 mL vial of Wash Buffer Concentrate to a final volume of 750 mL with deionized or distilled water.

Prepare 1X Tris Buffer

Dilute the contents of one vial of Tris Buffer Concentrate with 90 mL of deionized or distilled water.

Note: Concentrated buffer commonly contains crystalline salts after thawing. Rinse the vial to obtain any precipitated salts.

Prepare DEA Buffer

Dilute the 2.5 mL vial of DEA Buffer Concentrate to a final volume of 25 mL with deionized or distilled water.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Avoid freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) before analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Purify samples using the following procedure. This protocol works well for most samples, but may need optimization for sufficient purification of PGE_2 from all samples.

1. Split samples into two equal parts and place each sample into clean test tubes.
2. Spike one of these sets of samples with PGE_2.
3. Perform the following steps on all samples:
   a. Acidify the sample to pH <4.0 by the addition of 1.0 M HCl.
   b. Prepare a SPE C18 cartridge by conditioning it first with 5 mL of methanol followed by 5 mL distilled or deionized water.
   c. Apply the sample and allow to flow through the column.
   d. Rinse the column with 5 mL distilled or deionized water, followed by 5 mL hexane. Allow the column to dry following the hexane wash.
   e. Elute the PGE_2 with 5 mL ethyl acetate containing 1% methanol. If unable to run the assay at this time, store the samples in ethyl acetate/methanol at 80°C.
   f. Dry the sample under a stream of nitrogen. Reconstitute sample in 1X Tris Buffer. Assay unspiked and spiked samples with the ELISA.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

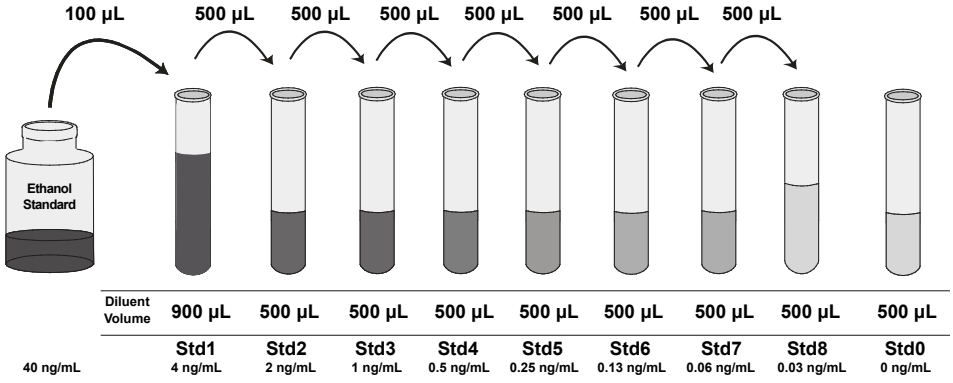
- Dilute samples that are greater than the highest standard point and retest.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: If assaying culture medium samples that have not been diluted with 1X Tris Buffer, the culture medium rather than 1X Tris Buffer should be used for dilution of the standard curve.

1. Equilibrate a pipette tip in ethanol, by repeatedly filling and expelling, then transfer 200 µL of the Human Prostaglandin E_2 standard into a clean test tube. Dilute with 300 µL distilled or deionized water. Label as 40 ng/mL Human Prostaglandin E_2.
2. Add 100 µL of 40 ng/mL Human Prostaglandin E_2 to one tube containing 900 µL 1X Tris buffer and mix. Label as 4 ng/mL Human Prostaglandin E_2.
3. Add 500 µL of 1X Tris Buffer to each of 8 tubes labeled as follows: 2, 1, 0.5, 0.25, 0.13, 0.06, 0.03, and 0 ng/mL Human Prostaglandin E_2.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. These diluted standards should be used within 24 hours. Diluted standards may be stored at 2 to 8°C for up to 24 hours.



Prepare Prostaglandin E_2 AP Tracer

Reconstitute the PGE_2 Alkaline Phosphatase Tracer with 6 mL of 1X Tris buffer. Vortex to mix. Store this reconstituted tracer at 2 to 8°C and use within 4 weeks.

Prepare Prostaglandin E_2 Monoclonal Antibody

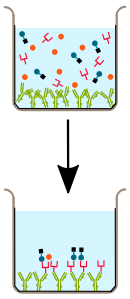
Reconstitute the PGE_2 Monoclonal Antibody with 6 mL of 1X Tris Buffer. Vortex to mix. Store this reconstituted antiserum at 2 to 8°C and use within four weeks.

Perform ELISA (Total assay time: 3.5 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



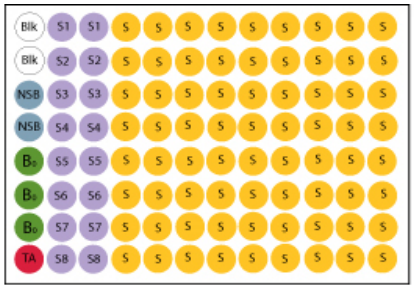


Fig. 1 Suggested Plate Plan

a. Add 150 µL 1X Tris Buffer into non-specific binding (NSB) wells, and 100 µL 1X Tris Buffer into zero standard (B_0) wells. If tissue culture medium was used to dilute the standard, substitute 100 µL of this same medium for the Tris Buffer in the NSB and B_0 wells.

b. Add 100 µL of standards and samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.

c. Add 50 µL PGE_2Alkaline Phosphatase Tracer into each well. Leave the wells for chromogen blanks empty, as well as total activity (TA) wells.

d. Add 50 µL of PGE_2 Antibody into each well. Leave the wells for chromogen blanks empty, as well as TA and NSB wells.

Note: The table below specifies reagents required for designated wells. Reading across the table, confirm you added the correct reagent(s) to each well

Well	Tris Buffer	Std/Sample	Tracer	Antibody
Blank	—	—	—	—
TA (Total Activity)	—	—	5 µL (at development)	—
NSB	150 µL	—	50 µL	—
B_0	100 µL	—	50 µL	50 µL
Std/Sample	—	100 µL	50 µL	50 µL

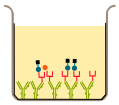
e. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate 2 hours at room temperature on an orbital shaker.

f. Dissolve 5 pNPP tablets in 25 mL 1x DEA buffer (25 mL is sufficient to develop 100 wells).

Note: Reconstituted pNPP is not stable, make only the amount needed at any given time.

g. Thoroughly aspirate the solution and wash wells 5 times with 1X Wash Buffer.

2 Add chromogen



a. Add 200 µL pNPP solution to each well. The substrate solution begins to turn yellow.

b. Add 5 µL of Alkaline Phosphatase Tracer to total activity (TA) wells.

c. Incubate for 60 to 90 minutes at room temperature in the dark.

Read the plate and generate the standard curve

1. Wipe the bottom of the plate and remove the plate cover.
2. Read the absorbance between 405 and 420 nm. Read plate within 2 hours of assay completion.
3. Use curve-fitting software to generate the standard curve. A 4-parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting. Alternatively, a spreadsheet program can be used. The data should be plotted as $\%B/B_0$ versus log concentration of standard using either a 4-parameter logistic or a logit curve fit . The following procedure is recommended to prepare the data prior to graphing:
   a. Prepare the data
      1. Subtract the absorbance of the blank wells from all wells on the plate.
      2. Average the absorbance readings from the NSB wells and B_0 wells.
      3. Subtract the average NSB from the average B_0. This is the corrected B_0.
      4. Calculate the $\%B/B_0$ for each standard and sample. To do this, subtract the average NSB absorbance from the standard and sample absorbances and divide by the corrected B_0 (from the previous step). Multiply by 100 to obtain $\%B/B_0$. Repeat for all wells.

$$\%B/B_0 = \left[\frac{\text{Absorbance - Average NSB}}{\text{Corrected } B_0}\right] \times 100$$

4. Plot $\%B/B_0$ for all standards versus PGE_2 concentration using log (x) and linear (y) axes, and fit the data to a four parameter logistic equation. Alternatively, the data can be linearized using a logit transformation (Logit $(B/B_0) = \ln [B/B_0 /(1-B/B_0)]$). Plot the data as logit (B/B_0) versus log concentration of standard and perform a linear regression fit

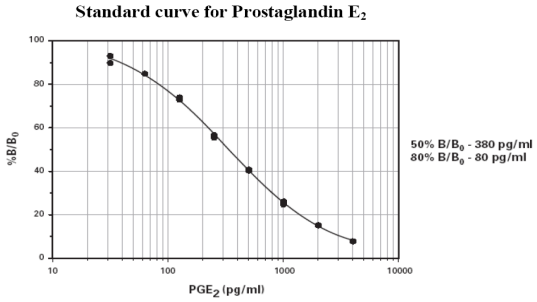
   Note: Do not use $\%B/B_0$ in this calculation
5. Calculate the $\%B/B_0$ for each sample. Determine the concentration of each sample using the equations obtained from the analysis of the standard curve. Remember to account for any dilution made to the sample prior to addition to the well. $\%B/B_0$ values of greater than 80% or less than 20% should be re-assayed as they generally fall outside of the linear range of the standard curve
6. If purification was performed, correct for recovery by dividing the concentration determined in step 4 by the recovery factor. Correct for any volume changes of the sample which may have occurred during purification.

$$\text{Recovery Factor} = \frac{\text{ELISA value of spiked (pg/mL) – ELISA value of unspiked sample (pg/mL)}}{\text{Concentration of spike (pg/mL)}}$$

Performance characteristics

Standard curve example

The standard curve is an example of data typically produced by this kit. Your results will vary from these, and it is therefore important that you run a standard curve each time you use the kit.



Intra-assay precision

Intra-assay precision was determined by measuring samples containing low, medium, and high concentrations of PGE2 multiple times in the same assay (8 samples per plate on a total of 5 plates).

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1,000	500	125
Standard Deviation	—	—	—
% Coefficient of Variation	3.5	4.5	9.9

Inter-assay precision

Inter-assay precision was determined by measuring low, medium, and high concentrations of the samples in eight separate assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1,000	500	125
Standard Deviation	—	—	—
% Coefficient of Variation	9.3	9.8	15.9

Specificity

Buffered solutions of a panel of substances were assayed. The following substances were tested and their corresponding cross-reactivity percentages are noted in the Cross-reactivity topic.

Cross-reactivity

The following substances were tested and their corresponding cross reactivity percentages are noted.

Analyte	Cross-Reactivity	Analyte	Cross-Reactivity
Prostaglandin E2	100%	Prostaglandin B2	<0.01%
Prostaglandin E2 Ethanolamide	100%	Prostaglandin D2	<0.01%
Prostaglandin E3	43.0%	15-keto Prostaglandin E2	<0.01%
8-iso Prostaglandin E2	37.4%	Prostaglandin F1a	<0.01%
Prostaglandin E1	18.7%	Prostaglandin F2a	<0.01%
6-keto Prostaglandin F1a	1.0%	13, 14-dihydro-15-keto Prostaglandin F2a	<0.01%
8-iso Prostaglandin F2a	0.25%	Prostaglandin F3a	<0.01%
Prostaglandin A1	<0.01%	Thromboxane B2	<0.01%
Prostaglandin A2	<0.01%	Tetranor PGEM	<0.01%
Prostaglandin A3	<0.01%	Tetranor PGFM	<0.01%
Prostaglandin B1	<0.01%	—	—

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.