



STAT-8-Isoprostane ELISA Kit

Item No. 500431

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
400432	STAT-8-Isoprostane ELISA Antiserum	1 vial/100 dtn	1 vial/500 dtn
400430	STAT-8-Isoprostane AP Tracer	1 vial/100 dtn	1 vial/500 dtn
400434	STAT-8-Isoprostane ELISA Standard	1 vial	1 vial
400080	Tris Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml
411007	AP Wash Buffer Concentrate (150X)	1 vial/5 ml	1 vial/12.5 ml
400082	DEA Buffer Concentrate (10X)	1 vial/2.5 ml	1 vial/12.5 ml
400004/400006	Mouse Anti-Rabbit IgG Coated Plate	1 plate	5 plates
400012	96-Well Cover Sheet	1 cover	5 covers
400090	pNPP Tablets	1 vial/5 ea	1 vial/25 ea
400040	ELISA Tracer Dye	1 vial	1 vial
400042	ELISA Antiserum Dye	1 vial	1 vial

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's ELISA Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

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

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 405-420 nm.
2. An orbital microplate shaker
3. Adjustable pipettes and a repeating pipettor.
4. A source of ultrapure water, with a resistivity of 18.2 MΩ.cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 11).

Description of AP Competitive ELISAs

This assay is based on the competition between 8-isoprostane and a 8-isoprostane alkaline phosphatase (AP) conjugate (8-isoprostane tracer) for a limited number of 8-isoprostane-specific rabbit antiserum binding sites. Because the concentration of the 8-isoprostane tracer is held constant while the concentration of 8-isoprostane varies, the amount of 8-isoprostane tracer that is able to bind to the 8-isoprostane polyclonal antiserum will be inversely proportional to the concentration of 8-isoprostane in the well. This antiserum-8-isoprostane complex binds to mouse monoclonal anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then *para*-nitrophenyl phosphate (*p*NPP) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 405 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of 8-isoprostane tracer bound to the well, which is inversely proportional to the amount of free 8-isoprostane present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound STAT-8-isoprostane AP Tracer}] \propto 1/[\text{8-isoprostane}]$$

A schematic of this process is shown below in Figure 2.

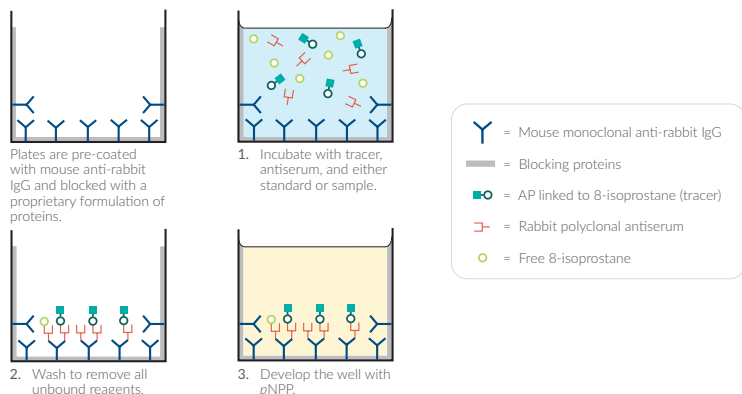


Figure 2. Schematic of the ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by *p*NPP. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including NSB wells.

TA (Total Activity): total enzymatic activity of the alkaline phosphatase-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B₀ (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B₀ (%Bound/Maximum Bound): ratio of the absorbance of a particular sample or standard well to that of the maximum binding (B₀) well.

Standard Curve: a plot of the %B/B₀ values *versus* concentration of a series of wells containing various known amounts of analyte.

Dtn (Determination): where one dtn is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B₀) value of the tested molecule to the mid-point (50% B/B₀) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\% \text{ Cross Reactivity} = \left[\frac{50\% \text{ B/B}_0 \text{ value for the primary analyte}}{50\% \text{ B/B}_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$

NOTE: 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. UltraPure water may be purchased from Cayman (Item No. 400000).

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for about two months.

1. Tris Buffer Preparation (1X)

Dilute the contents of one vial of Tris Buffer Concentrate (10X) (Item No. 400080) 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. AP Wash Buffer (1X) Preparation

5 ml vial AP Wash Buffer Concentrate (150X) (96-well kit; Item No. 411007): Dilute to a total volume of 750 ml with ultrapure water.

OR

12.5 ml vial AP Wash Buffer Concentrate (150X) (480-well kit; Item No. 411007): Dilute to a total volume of 1,875 ml with ultrapure water.

3. DEA Buffer (1X) Preparation

2.5 ml vial DEA Buffer Concentrate (10X) (96-well kit; Item No. 400082): Dilute to a total volume of 25 ml with ultrapure water.

OR

12.5 ml vial DEA Buffer Concentrate (10X) (480-well kit; Item No. 400082): Dilute to a total volume of 125 ml with ultrapure water.

Sample Preparation

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

In general, tissue culture supernatant samples may be diluted with Tris Buffer and added directly to the assay well. Plasma, serum, urine, whole blood, as well as other heterogeneous mixtures such as lavage fluids and aspirates often contain contaminants which can interfere in the assay. It is best to check for interference 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 within the linear portion of the standard curve (*i.e.*, 20-80% B/B₀). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated 8-isoprostane concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised.

Cayman offers an 8-Isoprostane Affinity Column and Affinity Sorbent (Item Nos. 401111 and 401113, respectively) which are recommended as the easiest and most convenient purification format for 8-isoprostane. The affinity column purification procedures have been validated with plasma and urine samples. Recoveries average >90% with a variance of <20%. Alternatively, SPE (solid phase extraction) method can be used for plasma purification (see protocol on page 19).

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C in the presence of 0.005% BHT (10 µl of 5 mg/ml solution in ethanol per 1 ml sample). Storage at -20°C is not sufficient to prevent oxidative formation of 8-isoprostane.⁹ BHT has limited solubility in water. Precipitate may form when BHT is added to aqueous solution.
- Samples of rabbit origin may contain antibodies that interfere with the assay by binding to the mouse anti-rabbit IgG-coated plate. We recommend that all rabbit samples be purified prior to use in this assay.

Lavage Fluids and Aspirates

Some lavage fluids may be assayed without purification. Samples that cannot be assayed immediately should be stored at -80°C in the presence of 0.005% BHT (see **General Precautions** above). Be certain to dilute the standards in the same medium as your samples. *NOTE: If you obtain inconsistent results, SPE or immunoaffinity purification is warranted.*

Culture Medium Samples

Most culture medium samples can be assayed without purification. Samples that cannot be assayed immediately should be stored at -80°C in the presence of 0.005% BHT (see **General Precautions** on page 12). If the estimated concentration in the samples is too low to allow dilution with Tris Buffer, be certain to dilute the 8-isoprostane standards in the same medium as the samples. *NOTE: If inconsistent results are obtained, SPE or immunoaffinity purification is warranted.*

Plasma

Plasma samples should be collected in vacutainers containing sodium citrate, heparin, or EDTA. Vacutainers can also be supplemented with indomethacin to give a final concentration of at least 10 μM . Indomethacin will prevent *ex vivo* formation of prostaglandins, which have the potential to interfere with this assay (although most prostaglandins do not appear to exhibit any cross reactivity (see page 34)). Samples that cannot be assayed immediately should be stored at -80°C in the presence of 0.005% BHT (see **General Precautions** on page 12).

Less than half of total plasma 8-isoprostane is present as the free acid, while the remainder is esterified in phospholipids.³ Direct ELISA of plasma samples without hydrolysis will measure only the free 8-isoprostane fraction. Total plasma 8-isoprostane determination requires an alkaline hydrolysis prior to ELISA (see page 18).

Analysis of plasma samples without purification may lead to inconsistent results. If inconsistent results are obtained, immunoaffinity purification is recommended as the easiest and most convenient purification format (see Figure 3, page 15).

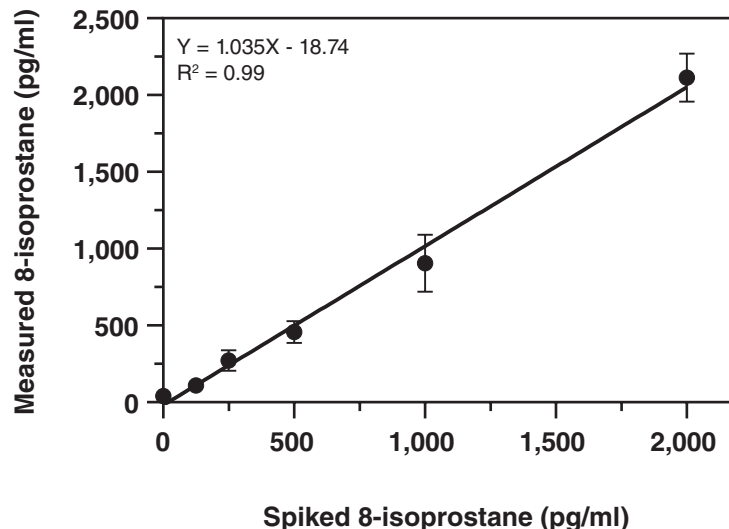


Figure 3. Spike and recovery of free 8-isoprostane in human plasma following SPE purification. Human plasma treated with 0.005%BHT was spiked with different amounts of 8-Isoprostane and serially diluted with Tris Buffer (1X), and evaluated using the STAT-8-Isoprostane ELISA Kit. The error bars represent standard deviations obtained from multiple dilutions of each sample.

Tissue

Tissue samples should be homogenized in 0.1 M Tris-HCl, pH 7.4, containing 1 mM EDTA and 0.005% BHT using a Polytron type homogenizer and then processed as described for plasma samples. As described in the **Plasma** section on page 14, most of the 8-isoprostane will be esterified in lipids, so hydrolysis must be performed in order to determine total amounts of 8-isoprostane.

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 Tris-HCl, pH 7.4, containing 1 mM EDTA and 0.005% BHT) 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. Cayman's Protein Determination Kit (Item No. 704002) may be used to normalize protein samples.

*NOTE: For total 8-isoprostane content, samples must be hydrolyzed. See **Free versus Total 8-Isoprostane Measurement**, on page 18, for the hydrolyzing procedure.*

Organ	Speed (rpm)	Cycle Length (seconds)	Beads
Lung	5,200	20	CK28 Large Ceramic (Item No. 10011151)
Brain	5,500	20	CK28 Large Ceramic (Item No. 10011151)
Liver	5,200	15	CK28 Large Ceramic (Item No. 10011151)
Kidney	5,200	20	CK14 Small Ceramic (Item No. 10011152)
Heart	5,200	30	CK14 Small Ceramic (Item No. 10011152)

Table 1. Precellys® settings

Sample Purification

Free versus Total 8-Isoprostane Measurement

Depending on the sample type, a large percentage of 8-isoprostane may be esterified in lipids within the sample and will not be detected by measurement of free 8-isoprostane. For the total 8-isoprostane measurement, it is necessary to perform a sample hydrolysis (procedure below), followed by sample purification. For the free 8-isoprostane measurement, proceed to either the **Preparation for Affinity Sorbent/Column Purification** section or the **Preparation for SPE Purification** section, on page 19. To hydrolyze samples:

1. Aliquot samples into clean glass tubes (300 μ l recommended).
2. Add an equal volume of 15% (w/v) KOH to all sample tubes.
3. Incubate at 37°C for 60 minutes.
4. Neutralize samples by the addition of approximately 600 μ l of 1N HCL to each sample. Ensure samples are at a neutral pH.
5. Proceed to either the **Preparation for Affinity Sorbent/Column Purification** section or the **SPE Purification Protocol** section on page 19.

Preparation for Affinity Sorbent/Column Purification

All samples must be free of particulates and precipitates to avoid plugging the column. This can be achieved either by filtration or by centrifugation. All samples must be at approximately neutral pH (6.5-7.5).

1. Urine samples should be centrifuged briefly to remove sediment and may be applied directly to the column or sorbent. Plasma samples should be diluted 1:5 with Eicosanoid Affinity Column Buffer and applied to the column or sorbent. Samples which have been hydrolyzed and then neutralized for measurement of total 8-isoprostane should be further diluted with 1/3 volume of Eicosanoid Affinity Column Buffer before being applied to the column or sorbent.
2. Proceed with purification following the protocol described in the product insert for the 8-isoprostane Affinity Sorbent, Column or Purification Kit (Item Nos. 401111, 401113, or 501110).

SPE Purification Protocol

1. Centrifuge samples at 1,000 x g for 10 minutes and transfer supernatant into a clean test tube. It will be used for SPE purification.
2. Activate a SPE cartridges (C-18) (6 ml) (Item No. 400020) by rinsing with 5 ml methanol and then with 5 ml ultrapure water. Do not allow the cartridge to become dry.
3. Pass sample through SPE cartridge. Rinse the cartridge with 5 ml ultrapure water. Allow the column to become dry after this step. Discard both washes.
4. Elute the 8-isoprostane with 2 ml of methanol.*
5. Add 300 μ l of Tris buffer (1X) and vortex. It is common for insoluble precipitate to remain after the addition of Tris buffer (1X); this will not affect the assay. The sample is now ready for use in the immunoassay.

**If it is necessary to stop during this purification, samples may be stored in methanol solution at -80°C.*

Preparation of Assay-Specific Reagents

STAT-8-Isoprostane ELISA Standard

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 100 μl of the STAT-8-Isoprostane ELISA Standard (Item No. 400434) into a clean test tube, then dilute with 900 μl of ultrapure water. The concentration of this solution (the bulk standard) will be 30 ng/ml.

NOTE: If assaying culture media samples that have not been diluted with Tris Buffer, culture medium should be used in place of Tris 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 Tris Buffer to tube #1 and 750 μl Tris Buffer to tubes #2-8. Transfer 100 μl of the bulk standard (30 ng/ml) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, will be 3 ng/ml (3,000 $\mu\text{g}/\text{ml}$). 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.

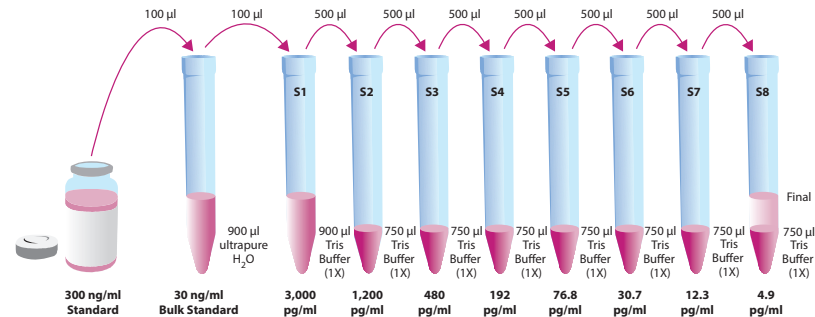


Figure 4. Preparation of the STAT-8-Isoprostane standards

STAT-8-Isoprostane AP Tracer

Reconstitute the STAT-8-Isoprostane AP Tracer as follows:

100 dtn STAT-8-Isoprostane AP Tracer (96-well kit; Item No. 400430):
Reconstitute with 6 ml Tris Buffer (1X).

OR

500 dtn STAT-8-Isoprostane AP Tracer (480-well kit; Item No. 400430):
Reconstitute with 30 ml Tris Buffer (1X).

Store the reconstituted STAT-8-Isoprostane AP 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). **NOTE: Do not store tracer with dye for more than 24 hours.**

STAT-8-Isoprostane ELISA Antiserum

Reconstitute the STAT-8-Isoprostane ELISA Antiserum as follows:

100 dtn STAT-8-Isoprostane ELISA Antiserum (96-well kit; Item No. 400432): Reconstitute with 6 ml Tris Buffer (1X).

OR

500 dtn STAT-8-Isoprostane ELISA Antiserum (480-well kit; Item No. 400432): Reconstitute with 30 ml Tris Buffer (1X).

Store the reconstituted STAT-8-Isoprostane ELISA Antiserum at 4°C (*do not freeze!*). It will be stable for at least four weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antiserum, if desired, to aid in visualization of antiserum-containing wells. Add the dye to the reconstituted antiserum at a final dilution of 1:100 (add 60 µl of dye to 6 ml antiserum or add 300 µl of dye to 30 ml of antiserum). **NOTE: Do not store antiserum with dye for more than 24 hours.**

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 are using a strip plate and do not 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 Blk, two NSB, and two B₀ wells, 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, assaying samples in triplicate is recommended.

A suggested plate format is shown in Figure 5, (see below). The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 29, **Analysis** for more details). It is recommended to you record the contents of each well on the template sheet provided (see page 38).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	1	1	9	9	9	17	17	17
B	Blk	S2	S2	2	2	2	10	10	10	18	18	18
C	NSB	S3	S3	3	3	3	11	11	11	19	19	19
D	NSB	S4	S4	4	4	4	12	12	12	20	20	20
E	B ₀	S5	S5	5	5	5	13	13	13	21	21	21
F	B ₀	S6	S6	6	6	6	14	14	14	22	22	22
G	B ₀	S7	S7	7	7	7	15	15	15	23	23	23
H	TA	S8	S8	8	8	8	16	16	16	24	24	24

Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
B₀ - Maximum Binding
S1-S8 - Standards 1-8
1-24 - Samples

Figure 5. Sample plate format

Performing the Assay

Pipetting Hints

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

Addition of the Reagents

1. Tris Buffer (1X)

Add 100 µl Tris Buffer (1X) to NSB wells. Add 50 µl Tris Buffer (1X) to B₀ wells. If culture medium was used to dilute the standard curve, substitute 50 µl of culture medium for Tris Buffer (1X) in the NSB and B₀ wells (i.e., add 50 µl culture medium to NSB and B₀ wells and 50 µl Tris Buffer to NSB wells).

2. STAT-8-Isoprostane ELISA Standard

Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl 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 should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. Samples

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

4. STAT-8-Isoprostane AP Tracer

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

5. STAT-8-Isoprostane ELISA Antiserum

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

Well	Tris Buffer	Standard/Sample	Tracer	Antiserum
Blk	-	-	-	-
TA	-	-	5 μ l (at devel. step)	-
NSB	100 μ l	-	50 μ l	-
B ₀	50 μ l	-	50 μ l	50 μ l
Std/Sample	-	50 μ l	50 μ l	50 μ l

Table 2. Pipetting summary

Incubation of the Plate

Cover each plate with the 96-Well Cover Sheet (Item No. 400012) and incubate one hour at room temperature on an orbital shaker.

Development of the Plate

1. When ready to develop the plate(s), reconstitute 5 pNPP tablets in 25 ml DEA Buffer (25 ml is sufficient to develop 100 wells). Reconstituted pNPP is not stable, so it is recommended that you store the reconstituted pNPP for no more than 24 hours. If the entire plate is not used at once, dissolve just enough pNPP for the number of wells used.
2. Empty the wells and rinse five times with Wash Buffer (1X).
3. Add 200 μ l of pNPP solution to each well.
4. Add 5 μ l of tracer to the TA wells.
5. Cover the plate with plastic film. Optimum development is obtained by using 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.*, B₀ wells \geq 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 pNPP solution has not splashed up on the plate cover. *NOTE: Any loss of pNPP will affect the absorbance readings. If pNPP is present on the cover, use a pipette to transfer the pNPP into the well.*
3. Read the plate at a wavelength between 405 nm. The absorbance may be checked periodically until the B_0 wells have reached a minimum of 0.6 A.U. (blank subtracted). The plate should be read when the absorbance of the B_0 wells are in the range of 0.5-0.8 A.U. (blank subtracted).

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 or as logit B/B_0 versus log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

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

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells 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 the remaining 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-parameter fit, multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool; the corrected B_0 divided by the actual TA (10X measured absorbance) will give the %Bound. This value should closely approximate the %Bound that can be calculated from the Sample Data (see page 31). Erratic absorbance values and a low (or no) %Bound could indicate the presence of organic solvents in the buffer or other technical problems (see page 35 for Troubleshooting).

Plot the Standard Curve

Plot %B/B₀ for standards S1-S8 versus 8-isoprostane concentration using linear (y) and log (x) axes and perform a four-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B₀ in this calculation.*

$$\text{logit (B/B}_0\text{)} = \ln [\text{B/B}_0\text{}/(1 - \text{B/B}_0\text{])}]$$

Plot the data as logit (B/B₀) versus log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B₀ (or %B/B₀) value for each sample. Determine the concentration of 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 the addition to the well. Samples with %B/B₀ values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.*

Performance Characteristics

Linearity

Plasma and serum samples spiked with 2,000 pg/ml and 250 pg/ml 8-isoprostane, respectively, were serially diluted with Tris Buffer (1X) and evaluated for linearity using the STAT-8-Isoprostane ELISA Kit. The results are shown in the table below.

Dilution	Concentration (pg/ml)	Dilutional Linearity (%)
Plasma		
4	2,056	100
8	2,258	110
16	1,947	94.7
32	2,133	104
Serum		
1	318	100
2	300	94.6
4	286	90.1

Table 3. Linearity in plasma and serum

*NOTE: Linearity has been calculated using the following formula: %Linearity = (Observed concentration value, dilution adjusted / First observed concentration value in the dilution series, dilution adjusted)*100*

Sample Data

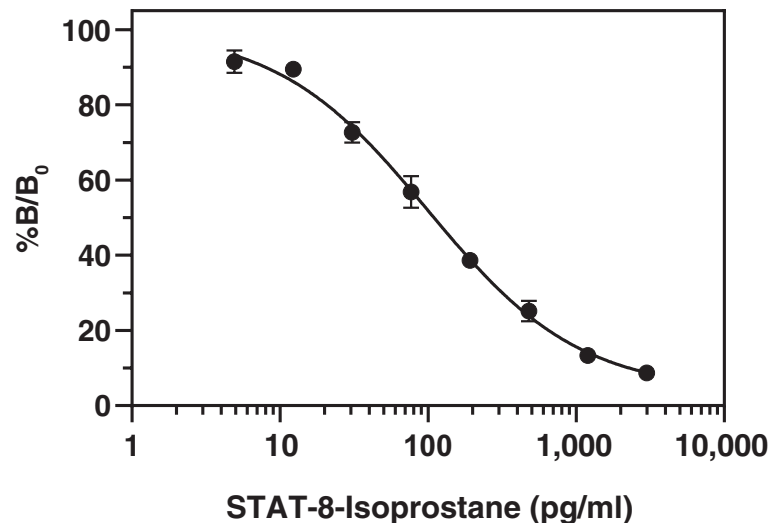
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the value of your samples. Your results could differ substantially.

STAT-8-Isoprostane (pg/ml) and Controls	Blk Subtracted Absorbance	NSB Corrected Absorbance	%B/B ₀	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
TA	0.471	--	--	--	--
NSB	0.006	--	--	--	--
B ₀	0.608	0.602	--	--	--
3,000	0.064	0.058	9.6	16.7	34.9
1,200	0.084	0.078	12.9	11.9	5.3
480	0.155	0.149	24.7	14.6	11.2
192	0.223	0.217	36.0	12.8	4.9
76.8	0.345	0.339	56.8	12.1	9.6
30.7	0.455	0.449	74.6	19.2	13.7
12.3	0.506	0.5	83.0	36.4**	19.7
4.9	0.556	0.55	91.4	52.6**	32.7

Table 4. Typical results

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

** Evaluate data in this range with caution



Assay Range = 4.9-3,000 pg/ml

Sensitivity (defined as 80% B/B₀) = 29 pg/ml

Mid-point (defined as 50% B/B₀) = 118 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with Tris Buffer (1X).

Figure 6. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of two human EDTA plasma controls in a single assay.

Matrix Control (pg/ml)	%CV
500	10.1
100	9.1

Table 5. Intra-assay precision

Inter-Assay precision was determined by analyzing two human EDTA plasma controls in eight separate assays on three different days.

Matrix Control (pg/ml)	%CV
500	19.7
100	24.8

Table 6. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity	Compound	Cross Reactivity
8-Isoprostane	100%	8-iso Prostaglandin F _{1β}	0.08%
8-iso Prostaglandin F _{3α}	20.6%	Thromboxane B ₂	0.08%
2,3-dinor-8-iso Prostaglandin F _{2α}	4.0%	11-dehydro Thromboxane B ₂	0.07%
8-iso Prostaglandin E ₂	1.84%	11β-Prostaglandin F _{2α}	0.03%
2,3-dinor-8-iso Prostaglandin F _{1α}	1.70%	PGE ₂	0.02%
8-iso Prostaglandin E ₁	1.56%	8-iso-15(R)-Prostaglandin F _{2α}	0.02%
Prostaglandin F _{1α}	0.71%	8,12-epi iPF _{2α} -III	0.01%
Prostaglandin F _{3α}	0.66%	iPF _{2α} -VI	<0.01%
Prostaglandin E ₁	0.39%	8,12-epi iPF _{2α} VI	<0.01%
Prostaglandin D ₂	0.16%	tetranor-PGEM	<0.01%
6-keto Prostaglandin F _{1α}	0.14%	tetranor-PGFM	<0.01%
Prostaglandin F _{2α}	0.14%	13,14-dihydro-15-keto Prostaglandin F _{2α}	<0.01%
2,3-dinor-6-keto Prostaglandin F _{1α}	0.09%		

Table 7. Cross Reactivity of the STAT-8-Isoprostane ELISA Antiserum

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	Replace activated carbon filter or change source of ultrapure water
High NSB (>10% of B ₀)	A. Poor washing B. Exposure of NSB wells to specific antibody	Rewash plate and redevelop
Very low B ₀	A. Trace organic contaminants in the water source B. Plate requires additional development time C. Dilution error in preparing reagents	A. Replace activated carbon filter or change source of ultrapure water B. Return plate to shaker and re-read later
Low sensitivity (shift in dose-response curve)	Standard is degraded	Replace standard
Analyses of two dilutions of a biological sample do not agree (i.e., more than 20% difference)	Interfering substances are present	Purify sample prior to analysis by ELISA ⁹
Only TA wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of ultrapure water

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