11-dehydro Thromboxane B_2 ELISA Kit - Monoclonal

Item No. 519510

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
419510	11-dehydro Thromboxane B_2 AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
419512	11-dehydro Thromboxane B_2 Monoclonal Antibody	1 vial/100 dtn	1 vial/500 dtn
419514	11-dehydro Thromboxane B_2 ELISA Standard	1 vial/0.5 ml	1 vial/0.5 ml
419517	11-dehydro Thromboxane B_2 Assay Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	1 vial/12.5 ml
400035	Polysorbate 20	1 vial/3 ml	1 vial/3 ml
400009/400008	Goat Anti-Mouse IgG-Coated Plate	1 plate	5 plates
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
400040	ELISA Tracer Dye	1 ea	1 ea
400042	ELISA Antiserum Dye	1 ea	1 ea
400012	96-Well Cover Sheet	1 ea	5 ea

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.

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 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; multichannel or repeating pipettor recommended
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 13)

INTRODUCTION

Background

11-dehydro Thromboxane B_2 (11-dehydro TXB_2) is a metabolite of TXA_2, an eicosanoid produced from arachidonic acid by cyclooxygenases (COXs) that induces irreversible platelet aggregation and contraction of smooth muscle, including vascular and bronchial smooth muscle.[1-3] TXA_2 is rapidly and non-enzymatically hydrolyzed to TXB_2, which is then converted into 11-dehydro TXB_2.[4,5]

Although it has been common to estimate TXA_2 levels by measuring TXB_2, most of the TXB_2 measured in the plasma or urine of healthy individuals is due to *ex vivo* platelet activation or intra-renal production, respectively.[6,7] Measurement errors are compounded by the fact that normal concentrations of circulating TXB_2 are extremely low (1-2 pg/ml) and highly transient ($t_{1/2}$ = 5-7 minutes).[4,6,8]

To circumvent this problem, it is necessary to measure a metabolite that cannot be formed by platelets *ex vivo* or by the kidney. TXB_2 can be metabolized by one or more 11-dehydro TX dehydrogenase enzymes, including cytosolic aldehyde dehydrogenase, to form 11-dehydro TXB_2 or by peroxisomal β-oxidation to form 2,3-dinor TXB_2.[4,5,9] Infusion studies using TXB_2 have shown that although both metabolites are formed, 11-dehyro TXB_2 has a longer circulating half-life ($t_{1/2}$ = 45 minutes) than 2,3-dinor TXB_2 ($t_{1/2}$ = 15 minutes).[6,10] Therefore, measurement of 11-dehydro TXB_2 in plasma or urine will provide a time-integrated indication of TXA_2 production.

Although 11-dehydro TXB_2 can be found in substantial amounts in urine, this compound is also subject to further metabolism, including β-oxidation, resulting in the formation of 11-dehydro-2,3-dinor TXB_2, which is also found in substantial quantities in urine.[6,9,11] Djurup *et al.* demonstrated that an immunoassay that measures the sum of 11-dehydro TXB_2 and 11-dehydro-2,3-dinor TXB_2 correlated well with measurement of 11-dehydro TXB_2 alone by GC/MS in urine samples from healthy subjects before and after dosing with aspirin.[12] Studies in smokers have also demonstrated that urinary changes in 11-dehydro-2,3-dinor TXB_2 parallel changes in 11-dehydro TXB_2 levels.[13] Cayman's 11-dehydro TXB_2 ELISA Kit measures both 11-dehydro TXB_2 and 11-dehydro-2,3-dinor TXB_2.

There is evidence suggesting that 11-dehydro TXB_2 can be formed non-enzymatically in whole blood following platelet activation, so it is important to process plasma samples quickly to prevent artifactual formation of 11-dehydro TXB_2.[1,14]

About This Assay

Cayman's 11-dehydro TXB_2 ELISA Kit is a competitive assay that can be used for the quantification of 11-dehydro TXB_2 in plasma, serum, urine, and other sample matrices. The assay has a range of 15.6-2,000 pg/ml, with a midpoint (50% B/B_0) of 80-160 pg/ml, and a sensitivity (80% B/B_0) of approximately 34 pg/ml.

Principle of this Assay

This assay is based on the competition between free 11-dehydro TXB_2 and an 11-dehydro TXB_2-acetylcholinesterase (AChE) conjugate (11-dehydro TXB_2-AChE Tracer) for a limited number of 11-dehydro TXB_2-specific monoclonal antibody binding sites. Because the concentration of the 11-dehydro TXB_2-AChE Tracer is held constant while the concentration of free 11-dehydro TXB_2 varies, the amount of 11-dehydro TXB_2-AChE Tracer that is able to bind to the 11-dehydro TXB_2 Monoclonal Antibody will be inversely proportional to the concentration of free 11-dehydro TXB_2 in the well. The antibody-11-dehydro TXB_2 complex binds to the goat anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and 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 11-dehydro TXB_2-AChE Tracer bound to the well, which is inversely proportional to the amount of free 11-dehydro TXB_2 present in the well during the incubation, as described in the equation:

Absorbance $\propto$ [Bound 11-dehydro TXB_2-AChE Tracer] $\propto$ 1/[11-dehydro TXB_2]

A schematic of this process is shown in Figure 1, on page 9.

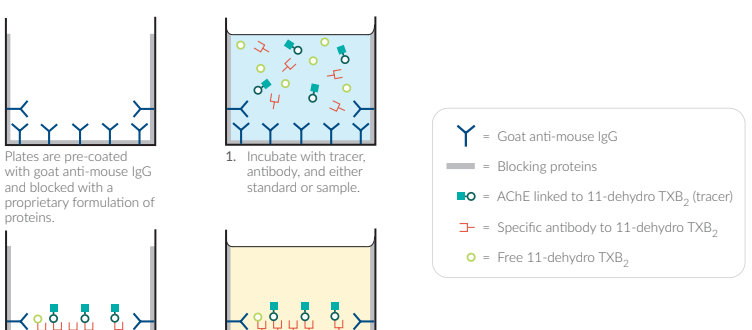


Figure 1. Schematic of the ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the 11-dehydro TXB_2 AChE-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.

B_0 (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a sample or standard well to the average absorbance of the maximum binding (B_0) wells.

Standard Curve: a plot of the %B/B_0 values *versus* concentration of a series of wells containing various known amounts of analyte.

Dtn (Determination): 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/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\text{\% Cross Reactivity} = \left[\frac{\text{50\% } B/B_0 \text{ value for the primary analyte}}{\text{50\% } B/B_0 \text{ value for the potential cross reactant}} \right] \text{x } 100\%$$

LLOD (Lower Limit of Detection): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

PRE-ASSAY PREPARATION

Buffer Preparation

NOTE: It is normal for the concentrated buffers to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.

1. **11-dehydro TXB_2 Assay Buffer (1X) Preparation**

   Dilute the contents of one vial of 11-dehydro TXB_2 Assay Buffer Concentrate (10X) (Item No. 419517) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

   Store diluted buffer at 4°C. It should be stable for at least two weeks.

2. **Wash Buffer (1X) Preparation**

   5 ml vial Wash Buffer Concentrate (400X) (96-well kit; Item No. 400062): Dilute to a total volume of 2 L with ultrapure water and add 1 ml of Polysorbate 20 (Item No. 400035) to make Wash Buffer (1X).

   OR

   12.5 ml vial Wash Buffer Concentrate (400X) (480-well kit; Item No. 400062): Dilute to a total volume of 5 L with ultrapure water and add 2.5 ml of Polysorbate 20 (Item No. 400035) to make Wash Buffer (1X).

   Store Wash Buffer (1X) at 4°C, where it will be stable for approximately two months.

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

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.

Sample Preparation

This assay has been validated in plasma, serum, and urine. Other sample types may also be used, but the purification protocols have not been fully tested.

Proper sample storage and preparation are essential for consistent and accurate results. Please read this section carefully before beginning the assay. *NOTE: 11-dehydro TXB_2 is capable of existing in two different conformations. The 11-dehydro TXB_2 Assay Buffer supplied with this kit is designed to convert all of the 11-dehydro TXB_2 into one conformation for more consistent results. All standards and samples should be diluted using this buffer (unpurified samples should be diluted at least 1:2).*

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.
- Samples of mouse or rat origin may contain antibodies that interfere with the assay by binding to the goat anti-mouse IgG-coated plate. We recommend that all mouse or rat samples be purified prior to use in the assay.

Testing for Interference

Plasma, serum, and urine as well as other heterogeneous mixtures, such as lavage fluids and aspirates, often contain contaminants that can interfere in immunoassays. It is best to check for interference before embarking on a large number of sample measurements. To test for interference, dilute one or two samples to obtain at least two different dilutions of each sample within the linear portion of the standard curve. If two different dilutions of the same sample show good correlation (differ by 20% or less) in the final calculated 11-dehydro TXB_2 concentration, sample purification is not required. If you do not see good correlation of the different dilutions, purification is advised. As described above, samples that are not purified should be diluted with 11-dehydro TXB_2 Assay Buffer (1X) at least 1:2.

Urine

It is strongly recommended that urine samples be diluted at least 1:2 into 11-dehydro TXB_2 Assay Buffer (1X) prior to testing in the assay. It is recommended that the values obtained from urine samples be standardized to creatinine levels using Cayman's Creatinine ELISA Kit (Item No. 502330), Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701), or a similar assay.

Plasma and Serum

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate for plasma samples and in vacutainers without a coagulant for serum samples. Indomethacin should be added immediately after whole blood collection (sufficient to give a 10 µM final concentration). Indomethacin will prevent *ex vivo* formation of eicosanoids, which have the potential to interfere with this assay (although most eicosanoids do not appear to exhibit any cross reactivity (see page 36)).[15] 11-dehydro TXB_2 may be formed non-enzymatically following platelet activation, so it is important to process plasma samples quickly to prevent artifactual formation of 11-dehydro TXB_2.[1,14]

To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes. Pipette off the top plasma layer without disturbing the white buffy layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

To obtain serum, allow samples to clot undisturbed for 30-60 minutes at room temperature. To remove the clot, centrifuge samples at 1-2,000 x g for 15-30 minutes. Pipette off the serum layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Acetone precipitation (see page 17) is recommended as a preparative step for plasma and serum sample analysis.

Processing Plasma and Serum: Acetone Precipitation

Plasma and serum were processed using the following protocol. Alternative protocols may be used based on the experimental requirements, sample type and the end user's expertise.

Add four volumes of ice cold acetone to plasma and serum samples and vortex briefly. Incubate for 15 minutes at 4°C and centrifuge at 5,000 x g. Transfer the supernatant into a clean test tube. Dry this acetone mixture under a stream of nitrogen, and reconstitute with a volume of 11-dehydro TXB_2 Assay Buffer (1X) equal to the original sample volume. If you wish to concentrate your samples, resuspend them in a volume of 11-dehydro TXB_2 Assay Buffer (1X) that is smaller than the original volume.

Sample Matrix Properties

Spike and Recovery

Plasma, serum, and urine were spiked with different amounts of 11-dehydro TXB_2, processed as described in the Sample Preparation section (see page 13), serially diluted with ELISA Buffer (1X), and evaluated using 11-dehydro TXB_2 ELISA Kit - Monoclonal. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

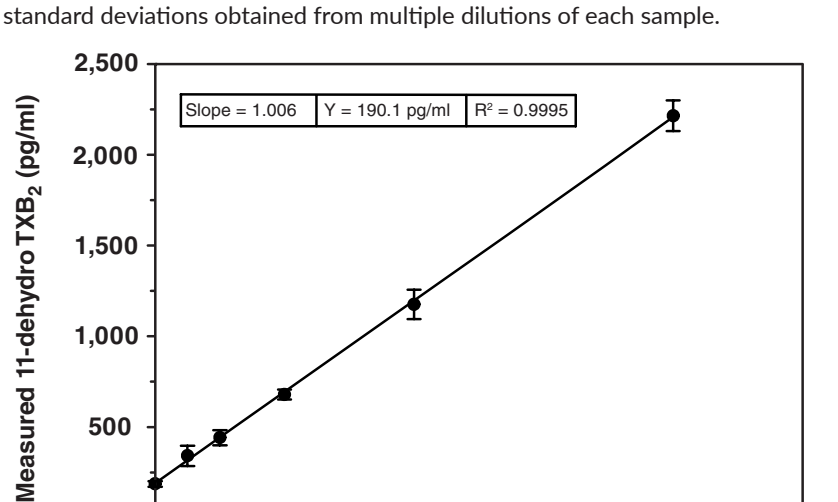


Figure 2. Spike and recovery of 11-dehydro TXB_2 in urine

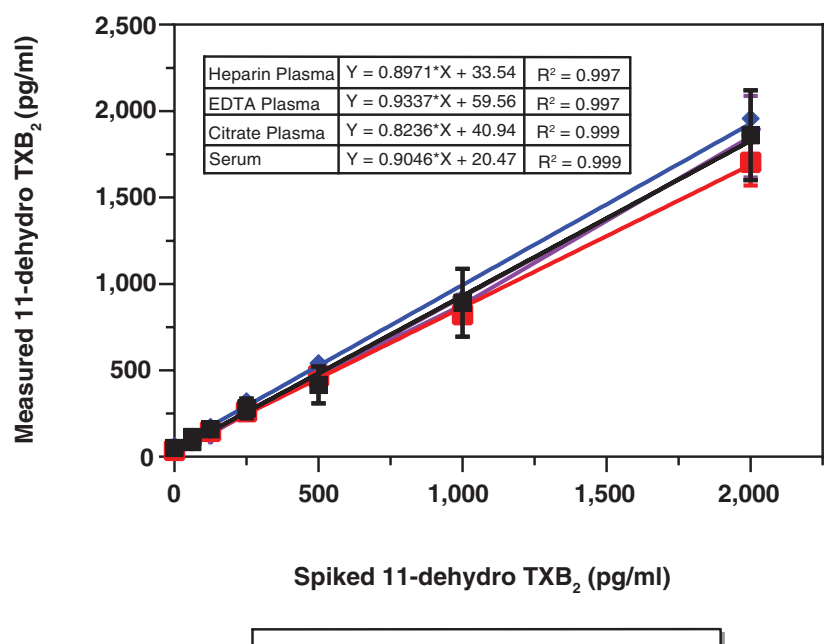


Figure 3. Spike and recovery of 11-dehydro TXB_2 in plasma and serum

Linearity

Plasma and urine samples were spiked with 2,000 pg/ml 11-dehydro TXB_2, processed as described in the Sample Preparation section, serially diluted with 11-dehydro TXB_2 Assay Buffer (1X), and evaluated for linearity using the 11-dehydro TXB_2 ELISA Kit - Monoclonal. The results are shown in the table below.

Dilution Factor	Measured Concentration (pg/ml)	Linearity (%)
Plasma (EDTA)		
8	1,927	100
16	2,003	104
32	1,942	101
Urine		
1	2,165	100
2	2,166	100
4	2,312	107

Table 1. Linearity in plasma and urine
*NOTE: Dilutional 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*

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

11-dehydro TXB_2 ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the 11-dehydro TXB_2 ELISA Standard (Item No. 419514) several times. Using the equilibrated pipette tip, transfer 100 µl of the standard into a clean test tube, then dilute with 900 µl of ultrapure water. The concentration of this solution (the bulk standard) will be 20 ng/ml. Store this bulk standard at 4°C where it will be stable for approximately six weeks.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 900 µl of 11-dehydro TXB_2 Assay Buffer (1X) to tube #1 and 500 µl of 11-dehydro TXB_2 Assay Buffer (1X) to tubes #2-8. Transfer 100 µl of the bulk standard (20 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place into tube #3; mix thoroughly. Repeat this process for tubes #4-8. Incubate these standards at room temperature for a minimum of two hours before adding to the plate. Alternatively, the standards and samples can be diluted with 11-dehydro TXB_2 Assay Buffer (1X) and incubated overnight at 4°C. On the following day, allow all reagents to equilibrate to room temperature prior to use, and follow the protocol in the **Performing the Assay** section (see page 26). The diluted standards should not be stored at 4°C for more than 24 hours.

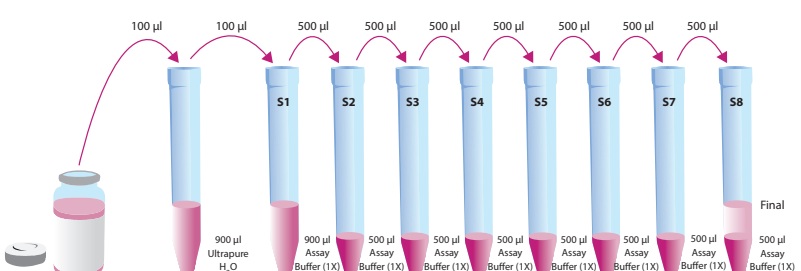


Figure 4. Preparation of the 11-dehydro TXB_2 standards

11-dehydro TXB_2-AChE Tracer

Reconstitute the 11-dehydro TXB_2-AChE Tracer (Item No. 419510) as follows:

100 dtn 11-dehydro TXB_2-AChE Tracer (96-well kit): Reconstitute with 6 ml of 11-dehydro TXB_2 Assay Buffer (1X).

OR

500 dtn 11-dehydro TXB_2-AChE Tracer (480-well kit): Reconstitute with 30 ml of 11-dehydro TXB_2 Assay Buffer (1X).

The tracer may be used immediately following reconstitution. Store the reconstituted 11-dehydro TXB_2-AChE Tracer at 4°C *(do not freeze!)*. It will be stable for two 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.*

11-dehydro TXB_2 Monoclonal Antibody

Reconstitute the 11-dehydro TXB_2 Monoclonal Antibody (Item No. 419512) as follows:

100 dtn 11-dehydro TXB_2 Monoclonal Antibody (96-well kit): Reconstitute with 6 ml of 11-dehydro TXB_2 Assay Buffer (1X).

OR

500 dtn 11-dehydro TXB_2 Monoclonal Antibody (480-well kit): Reconstitute with 30 ml of 11-dehydro TXB_2 Assay Buffer (1X).

Store the reconstituted 11-dehydro TXB_2 Monoclonal Antibody at 4°C *(do not freeze!)*. It will be stable for at least two 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). *NOTE: Do not store antiserum with dye.*

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 Blk, two NSB, and three B_0 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 a minimum of 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 5, 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 30 for more details). We suggest recording the contents of each well on the template sheet provided (see page 41).

	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
B_0 - 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. **11-dehydro TXB_2 Assay Buffer**

   Add 100 µl 11-dehydro TXB_2 Assay Buffer (1X) to NSB wells. Add 50 µl 11-dehydro TXB_2 Assay Buffer (1X) to B_0 wells.

2. **11-dehydro TXB_2 ELISA Standard**

   After the standards have been diluted and incubated at room temperature for at least two hours (or for optional overnight 4°C incubation) they may be added to the plate. 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. **11-dehydro TXB_2-AChE Tracer**

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

5. **11-dehydro TXB_2 Monoclonal Antibody**

   Add 50 µl to each well *except* the TA, NSB, and Blk wells within 15 minutes of addition of the tracer.

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate two hours at room temperature on an orbital shaker.

Development of the Plate

1. Reconstitute Ellman's Reagent (Item No. 400050) immediately before use (20 ml of reagent is sufficient to develop 100 wells). Reconstitute 100 dtn vial with 20 ml of ultrapure water. Reconstitute 250 dtn vial with 50 ml of ultrapure water.

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

2. Empty the wells and rinse five times with Wash Buffer (1X).
3. Add 200 µl of Ellman's Reagent to each well.
4. Dilute the 11-dehydro TXB_2-AChE Tracer 1:10 with 11-dehydro TXB_2 Assay Buffer (1X) (*e.g.*, 50 µl tracer into 450 µl assay buffer).
5. Add 5 µl of the diluted tracer to the TA wells.
6. Cover the plate with the 96-Well Cover Sheet. 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.*, B_0 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 cover sheet being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings.*
3. Read the plate at a wavelength between 405 and 420 nm (usually 412 nm). The absorbance may be checked periodically until the B_0 wells have reached a minimum of 0.3 A.U. (Blk subtracted). The plate should be read when the absorbance of the B_0 wells is in the range of 0.3-1.5 A.U. (Blk subtracted). If the absorbance of the wells exceeds 1.5 A.U., wash the plate, add fresh Ellman's Reagent, and let it develop again.

ANALYSIS

Many plate readers come with data reduction software that plots 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 Blk 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. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system.

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* 11-dehydro TXB_2 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/B_0 in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

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 for each sample. Determine the concentration of each sample by identifying the %B/B_0 on the standard curve and reading the corresponding values on the x-axis. *NOTE: Remember to account for any dilution of the original sample concentration prior to its addition 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 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.

NOTE: If there is an error in the B_0 wells it is possible to calculate sample concentrations by plotting the absorbance values and back calculating sample absorbance off the standard curve.

Performance Characteristics

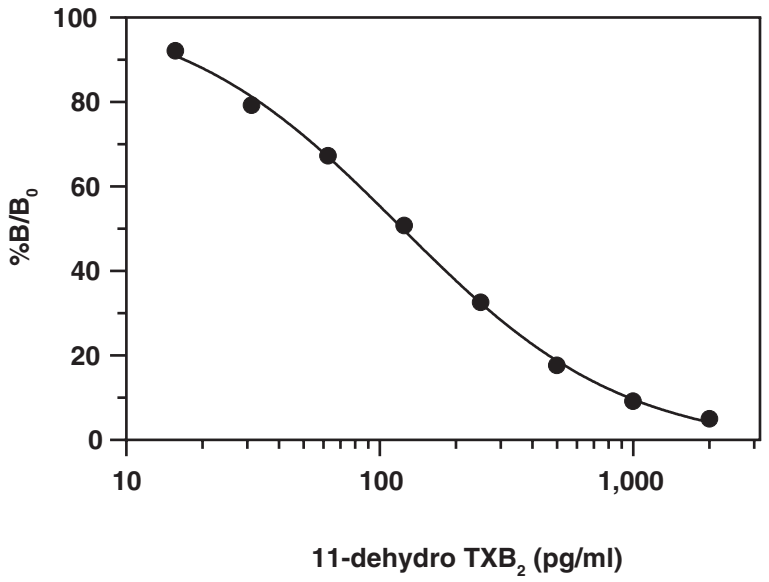
Representative Data

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 values of your samples.

Absorbance at 414 nm

11-dehydro TXB_2 Standards (pg/ml) and Controls	Blk-subtracted Absorbance	NSB-corrected Absorbance	$\%B/B_0$	%CV* Intra-assay Precision	%CV* Inter-assay Precision
NSB	0.001				
B_0	1.204	1.203			
TA	0.567				
2,000	0.061	0.060	5.0	5.6	5.0
1,000	0.112	0.111	9.2	5.2	3.4
500	0.214	0.213	17.7	2.9	2.1
250	0.395	0.394	32.7	4.5	2.1
125	0.612	0.611	50.8	5.0	3.1
62.5	0.811	0.810	67.3	5.2	3.0
31.3	0.955	0.954	79.3	9.8	4.0
15.6	1.110	1.109	92.2	14.4	4.1

Table 2. Typical results
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve



Assay Range = 15.6-2,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 34 pg/ml
Mid-point (defined as 50% B/B_0) = 120 pg/ml
LLOD = 16 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with 11-dehydro TXB_2 Assay Buffer (1X).

Figure 6. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 56 replicates of three urine controls in a single assy.

Matrix Control (pg/ml)	%CV
465	5.0
196	7.4
77	7.5

Table 3. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of three urine controls in four separate assays on different days.

Matrix Control (pg/ml)	%CV
453	6.0
175	12.9
69	11.7

Table 4. Inter-assay precision
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

Cross Reactivity:

Compound	Cross Reactivity
11-dehydro TXB_2	100%
*11-dehydro-2,3-dinor TXB_2	330%
Prostaglandin D_2	0.12%
2,3-dinor TXB_2	0.10%
TXB_2	0.08%
Arachidonic Acid	<0.01%
Prostaglandin $F_{2\alpha}$	<0.01%

Table 5. Cross reactivity of the 11-dehydro TXB_2 Monoclonal ELISA
*This assay measures both 11-dehydro TXB_2 and 11-dehydro-2,3-dinor TXB_2. The physiological revelance of these metabolites are similar.[11,12]

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (>10% B_0)	A. Poor washing B. Exposure of NSB wells to specific antibody
Very low B_0	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Low sensitivity (shift in dose-response)	A. Standard is degraded or contaminated B. Dilution error in preparing standards
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present
Low signal in the sample wells (below the range of the standard curve)	A. AChE inhibitors are present; ensure that the samples and buffers are free of AChE inhibitors B. Sample requires further dilution
Only TA wells develop	A. Trace organic contaminants in the water source B. The tracer was not added to the wells

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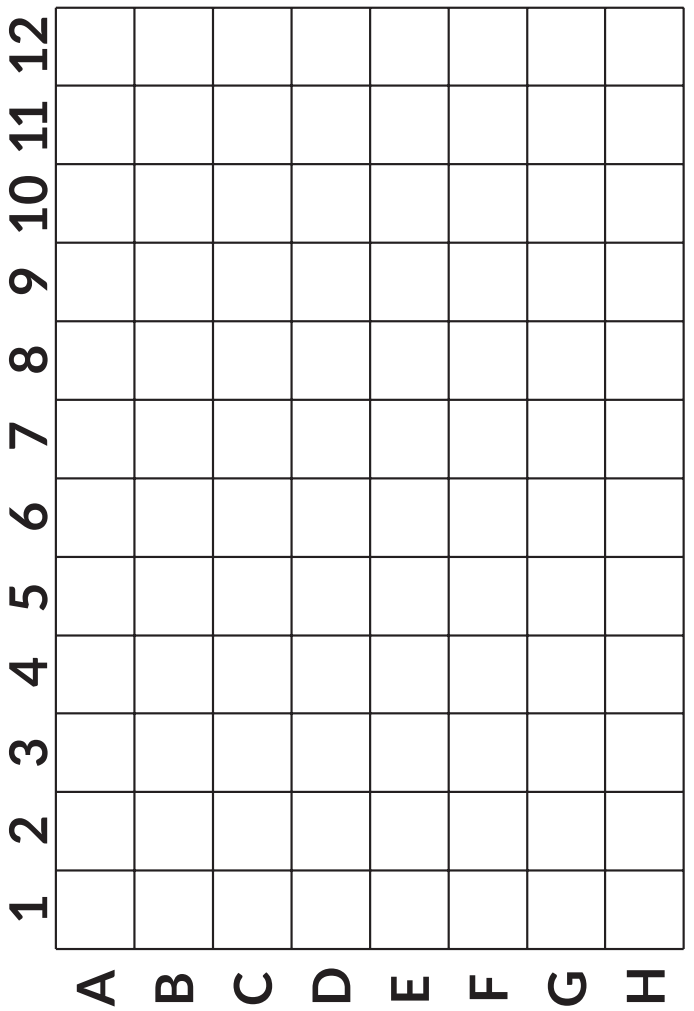
Procedure	Blk	TA	NSB	B_0	Standards/ Samples
Dilute or Reconstitute and Mix	Mix all reagents gently				
11-dehydro TXB_2 Assay Buffer (1X)	--	--	100 µl	50 µl	--
Standards/Samples	--	--	--	--	50 µl
11-dehydro TXB_2-AChE Tracer	--	--	50 µl	50 µl	50 µl
11-dehydro TXB_2 Monoclonal Antibody	--	--	--	50 µl	50 µl
Incubate	Seal the plate and incubate for two hours at room temperature on an orbital shaker				
Aspirate	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)				
Apply Ellman's Reagent	200 µl	200 µl	200 µl	200 µl	200 µl
TA - Apply Tracer	--	5 µl	--	--	--
Develop	Seal the plate and incubate 60-90 minutes at room temperature on orbital shaker protected from light				
Read	Read absorbance between 405-420 nm				

Table 6. Assay summary



NOTES

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