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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Leukotriene B_4 ELISA Kit

Item No. 502390

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
400511	Leukotriene B_4 ELISA Monoclonal Antibody	1 vial/100 dtn	1 vial/500 dtn
400512	Leukotriene B_4 AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
400513	Leukotriene B_4 ELISA Standard	1 vial/0.5 ml	1 vial/0.5 ml
400060	ELISA 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
400008/ 400009	Goat Anti-Mouse IgG-Coated Plate	1 plate	5 plates
400012	96-Well Cover Sheet	1 ea	5 ea
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

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 12)

INTRODUCTION

Background

Leukotriene B_4 (LTB_4) is synthesized from arachidonic acid by the combined action of 5-lipoxygenase and LTA_4 hydrolase.[1] LTB_4 has long been recognized as a potent mediator of inflammation. It binds to LTB_4 receptor 1 (BLT_1) and BLT_2, its high- and low-affinity G protein-coupled receptors (GPCRs), respectively.[2,3] LTB_4 stimulates a number of leukocyte functions, including aggregation, stimulation of ion fluxes, enhancement of lysosomal enzyme release, superoxide anion production, chemotaxis, and chemokinesis.[4,5] In subnanomolar ranges (3.9×10^{-10} M), LTB_4 induces chemotaxis and chemokinesis in human polymorphonuclear leukocytes.[4] At higher concentrations (1.0×10^{-7} M), LTB_4 induces neutrophil aggregation and degranulation, as well as superoxide anion production.[4,6] Plasma levels of LTB_4 increase from less than 100 pg/ml to greater than 100,000 pg/ml following leukocyte stimulation.[7-9] LTB_4 is metabolized in leukocytes and hepatocytes to less active 20-hydroxy- and 20-carboxy LTB_4 by NADPH-dependent cytochrome P450 enzymes followed by β-oxidation at the ω-end to ω-carboxy dinor LTB_4 and ω-carboxy tetranor-LTB_3.[10-13] LTB_4 is not excreted in the urine.[14]

About This Assay

Cayman's LTB_4 ELISA Kit is a competitive assay that can be used for the quantification of LTB_4 in plasma and other sample matrices. The assay has a range of 3.9-500 pg/ml, with a midpoint (50% B/B_0) of 30-60 pg/ml and an average sensitivity (80% B/B_0) of approximately 12 pg/ml.

Principle of this Assay

This assay is based on the competition between free LTB_4 and an LTB_4 acetylcholinesterase (AChE) conjugate (LTB_4 AChE Tracer) for a limited number of LTB_4 monoclonal antibody binding sites. Because the concentration of the LTB_4 AChE Tracer is held constant while the concentration of free LTB_4 varies, the amount of LTB_4 AChE Tracer that is able to bind to the LTB_4 monoclonal antibody will be inversely proportional to the concentration of free LTB_4 in the well. This antibody-LTB_4 complex binds to goat 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 LTB_4 AChE Tracer bound to the well, which is inversely proportional to the amount of free LTB_4 present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound } LTB_4 \text{ AChE Tracer}] \propto 1/[LTB_4]$$

A schematic of this process is shown in Figure 1, on page 8.

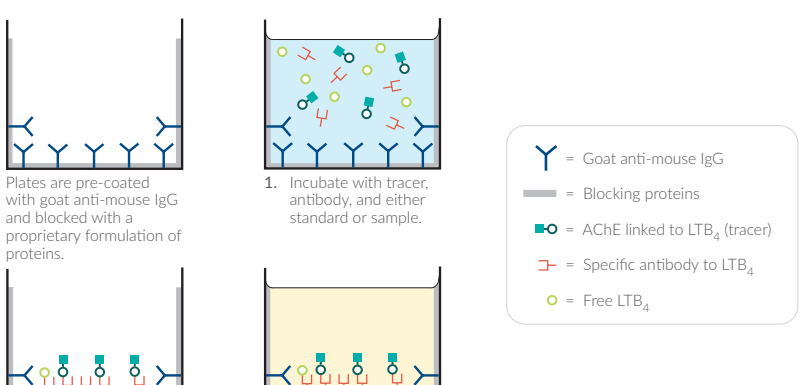


Figure 1. Schematic of the ELISA

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 LTB_4 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 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:

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

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

Store all diluted buffers at 4°C; they will be stable for about 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 (1X) Preparation**

   Dilute the contents of one vial of ELISA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

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).

   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).

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

In general, tissue culture supernatant samples may be diluted with ELISA Buffer (1X) and added directly to the assay well. Plasma, serum, and whole blood, as well as other heterogeneous mixtures such as cerebrospinal fluid (CSF), often contain contaminants that can interfere in the assay. It is best to check for interference before beginning a large number of sample measurements. To test for interference, dilute one or two samples to obtain at least two different dilutions 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 LTB_4 concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised.[12]

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 origin may contain antibodies that interfere with the assay by binding to the goat anti-mouse plate. We recommend that all mouse samples be purified prior to use in this assay.

Plasma

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate. 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 30)).[13]

Plasma/Serum Purification

Plasma/Serum can be purified using either the SPE (C-18) protocol or the Protein Precipitation Protocol below. Alternative protocols may be used based on the experimental requirements, sample type, and the end user's expertise.

Materials Needed

1. 1 M Acetate buffer (pH 4.0), deionized water, ethanol, methanol, and ethyl acetate
2. 500 mg SPE Cartridges (C-18). Available from Cayman (Item No. 400020)

SPE (C-18) Protocol

1. Aliquot a known amount of each sample into a clean test tube (500 µl is recommended). If the 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. 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.
3. Prepare SPE (C-18) columns by rinsing with 2 x 2.5 ml methanol followed by 2 x 2.5 ml deionized water. Do not allow the SPE cartridge to dry.
4. Apply the sample to the SPE cartridge (C-18) and allow the sample to completely enter the packing material.
5. Wash the column with 2 x 2.5 ml deionized water and evacuate any residual water by applying a slight positive pressure. Discard the wash.
6. Elute the LTB_4 from the column with 2 x 2.5 ml ethyl acetate containing 1% methanol. It may be necessary to start the flow by addition of a slight positive pressure. 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.

7. 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.
8. To resuspend the sample, add 500 µl ELISA Buffer (1X) and vortex. It is common for insoluble precipitate to remain in the sample after addition of ELISA Buffer (1X); this will not affect the assay. The sample is now ready for use in the ELISA.

Protein Precipitation Protocol

1. To precipitate proteins, add cold ethanol (approximately four times the sample volume) to each tube. Vortex to mix thoroughly.
2. Incubate samples at 4°C for five minutes, then centrifuge at 3,000 x g for 10 minutes to remove precipitated proteins.
3. Transfer the supernatant to a clean test tube and evaporate under nitrogen.
4. Resuspend the sample in ELISA Buffer (1X) to its original volume, and use this for ELISA analysis.

Sample Matrix Properties

Spike and Recovery

EDTA plasma samples were spiked with different amounts of LTB_4, purified by protein precipitation, as described in **Plasma/Serum Purification** section (on page 14), serially diluted with ELISA Buffer (1X), and evaluated using the Leukotriene B_4 ELISA kit. The error bars represent standard deviations obtained from multiple dilutions of each sample.

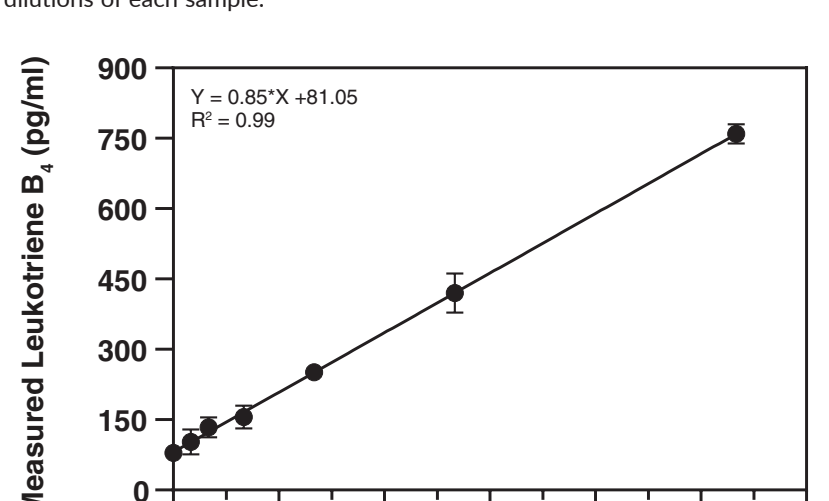


Figure 2. Spike and recovery of LTB_4 in plasma

Linearity

Human EDTA plasma samples were spiked with 800 or 400 pg/ml LTB_4, purified by protein precipitation (see page 14), serially diluted with ELISA Buffer (1X), and evaluated for linearity using the Leukotriene B_4 ELISA Kit. Results are shown in the table below.

Dilution Factor	Measured Concentration (pg/ml)	Linearity (%)
Spike: 800 pg/ml		
10	760.3	100
20	779.2	102
40	738.3	97.1
Spike: 400 pg/ml		
5	464.4	100
10	380.9	82.0
20	415.0	89.4

Table 1. Linearity in plasma

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*

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Leukotriene B_4 ELISA Standard

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with LTB_4 ELISA Standard (Item No. 400513). Using the equilibrated pipette tip, transfer 100 µl of the standard into a clean test tube, then dilute with 900 µl ultrapure water. The concentration of this solution (the bulk standard) will be 5 ng/ml.

NOTE: If assaying culture medium samples that have not been diluted with ELISA Buffer (1X), culture medium should be used in place of ELISA Buffer (1X) for dilution of the standard curve.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 900 µl ELISA Buffer (1X) to tube #1 and 500 µl ELISA Buffer (1X) to tubes #2-8. Transfer 100 µl of the bulk standard (5 ng/ml) to tube #1 and mix thoroughly. 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.

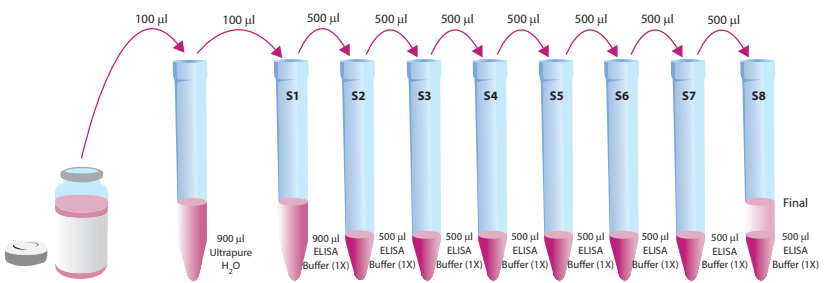


Figure 3. Preparation of the LTB_4 standards

Leukotriene B_4 AChE Tracer

Reconstitute the LTB_4 AChE Tracer as follows:

100 dtn LTB_4 AChE Tracer (96-well kit; Item No. 400512): Reconstitute with 6 ml ELISA Buffer (1X).

OR

500 dtn LTB_4 AChE Tracer (480-well kit; Item No. 400512): Reconstitute with 30 ml ELISA Buffer (1X).

Store the reconstituted LTB_4 AChE Tracer at 4°C *(do not freeze!)*. It will be stable for 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). Do not store the tracer with dye.

Leukotriene B_4 ELISA Monoclonal Antibody

Reconstitute the LTB_4 ELISA Monoclonal Antibody as follows:

100 dtn LTB_4 ELISA Monoclonal Antibody (96-well kit; Item No. 400511): Reconstitute with 6 ml ELISA Buffer (1X).

OR

500 dtn LTB_4 ELISA Monoclonal Antibody (480-well kit; Item No. 400511): Reconstitute with 30 ml ELISA Buffer (1X).

Store the reconstituted LTB_4 ELISA Monoclonal Antibody at 4°C. It will be stable for at least four weeks. A 20% surplus of monoclonal 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). Do not store the antibody 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 two 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 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 4, 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 24, for more details). We suggest recording you record the contents of each well on the template sheet provided (see page 33).

	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 Wells
TA = Total Activity Wells
NSB = Non-Specific Binding Wells
B_0 = Maximum Binding Wells
S1-S8 = Standard Wells
1-24 = Sample Wells

Figure 4. 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. **ELISA Buffer (1X)**

   Add 100 µl ELISA Buffer (1X) to NSB wells. Add 50 µl ELISA Buffer (1X) to B_0 wells. If culture medium was used to dilute the standard curve, substitute 50 µl of culture medium for ELISA Buffer (1X) in the NSB and B_0 wells (*i.e.*, add 50 µl culture medium to NSB and B_0 wells and 50 µl ELISA Buffer (1X) to NSB wells).

2. **Leukotriene B_4 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. **Leukotriene B_4 AChE Tracer**

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

5. **Leukotriene B_4 ELISA Monoclonal Antibody**

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

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate overnight at 4°C.

Development of the Plate

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

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

   OR

   250 dtn vial Ellman's Reagent (480-well kit; Item No. 400050): Reconstitute 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 ~300 µl of Wash Buffer (1X).
3. Add 200 µl of Ellman's Reagent to each well.
4. Add 5 µl of the reconstituted tracer to the TA wells.
5. 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 at room temperature. This assay typically develops (*i.e.*, B_0 wells ≥0.3 A.U. (blank subtracted)) in 90-120 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 the absorbance readings.*
3. Read the plate at a wavelength between 405-420 nm. The absorbance may be checked periodically until the B_0 wells have reached a minimum of 0.3 A.U. (blank subtracted). The plate should be read when the absorbance of the B_0 wells in the range of 0.3-2.0 A.U. (blank subtracted). If the absorbance of the wells exceeds 2.0, wash the plate, add fresh Ellman's Reagent, and let it develop again. Some spectrophotometers can accurately read absorbances higher than 2.0 A.U., but it is necessary to get confirmation from the instrument's manufacturer first.

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 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. 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* LTB_4 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 concentration of the sample prior to the 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: Although the B_0 wells are required, if there is an error in the B_0 wells, it is possible to calculate sample concentrations by plotting the standard absorbance values and calculating sample concentration off the standard curve using sample absorbance.

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. Your results could differ substantially.

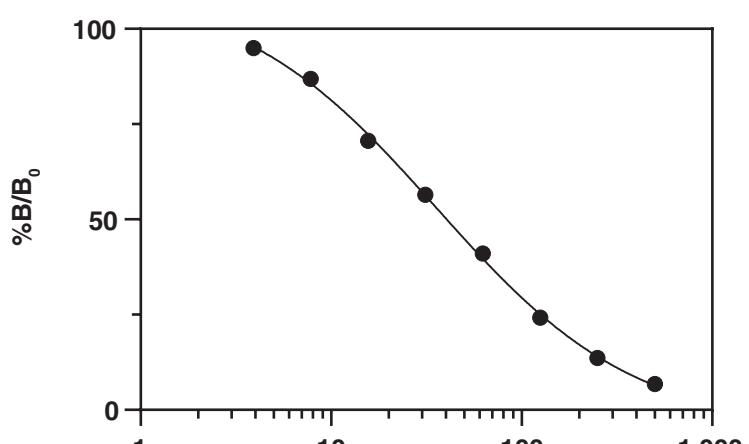
Absorbance at 414 nm (90 minutes)

LTB_4 (pg/ml) and Controls	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	$\%B/B_0$	*%CV Intra-Assay Precision	*%CV Inter-Assay Precision
TA	1.35	--	--	--	--
NSB	0.007	--	--	--	--
B_0	1.246	1.239	--	--	--
500	0.091	0.084	6.8	7.5	3.9
250	0.176	0.169	13.6	7.8	6.4
125	0.306	0.299	24.2	10.4	8.4
62.5	0.515	0.508	41.0	8.3	4.8
31.3	0.706	0.699	56.5	7.2	5.9
15.6	0.882	0.875	70.7	11.0	9.9
7.8	1.083	1.076	86.9	13.1	13.4
3.9	1.184	1.177	95.0	38.2**	8.7

Table 2. 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 = 3.9-500 pg/ml
Sensitivity (defined as 80% B/B_0) = 10.7 pg/ml
Mid-point (defined as 50% B/B_0) = 41.6 pg/ml
Lower Limit of Detection (LLOD) = 6.5 pg/ml

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

Figure 5. Typical standard curve

Precision:

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

Matrix Control (pg/ml)	%CV
800	18.4
400	9.7
100	10.2

Table 3. Intra-assay precision

Inter-assay precision was determined by analyzing three human heparin plasma controls in eight separate assays on four different days.

Matrix Control (pg/ml)	%CV
800	8.7
400	10.0
100	10.7

Table 4. Inter-assay precision

Sensitivity:

The Lower Limit of Detection (LLOD) of this assay is 6.5 pg/ml.

Cross Reactivity:

Compound	Cross Reactivity	Compound	Cross Reactivity
Leukotriene B_4	100%	Prostaglandin E_2	0.04%
Leukotriene B_5*	390%	Leukotriene D_4	<0.02%
20-hydroxy Leukotriene B_4	36%	5(S)-HETE	<0.01%
12-*epi* Leukotriene B_4	6.8%	5(R)-HETE	<0.01%
20-carboxy Leukotriene B_4	2.3%	15(R)-HETE	<0.01%
6-*trans* Leukotriene B_4	0.13%	15(S)-HETE	<0.01%
6-*trans*-12-*epi* Leukotriene B_4	0.09%	5,6-DiHETE	<0.01%
Resolvin D2	0.06%	Glutathione	<0.01%
Resolvin D1	0.05%	Leukotriene A_4	<0.01%
Arachidonic Acid	0.05%	Leukotriene C_4	<0.01%
Arachidonyl Ethanolamide	0.05%	Leukotriene E_4	<0.01%
Prostaglandin $F_{2\alpha}$	0.05%	19(R)-hydroxy Prostaglandin B_2	<0.01%
12(S)-HETE	0.04%		

Table 5. Cross reactivity of the LTB_4 ELISA
**Leukotriene B_5, derived from n-3 fatty acids, is mostly detectable in humans and animals with a diet rich in, or supplemented with, eicosapentaenoic acid.*[15]

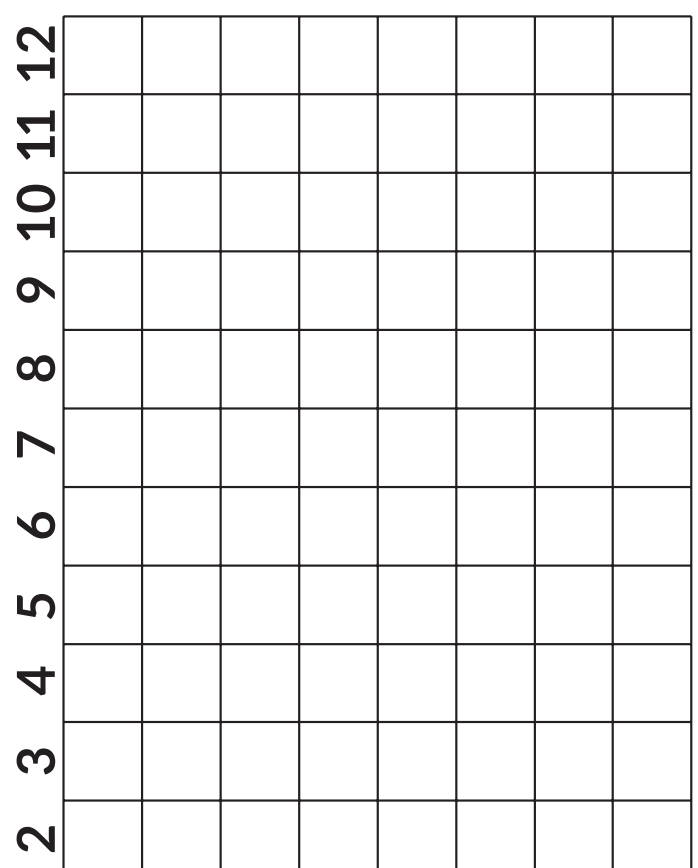
RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (>10% of 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 curve)	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

Procedure	Blk	TA	NSB	B_0	Standards/ Samples
Reconstitute and Mix	Mix all reagents gently				
ELISA Buffer (1X)	--	--	100 µl	50 µl	--
Standards/Samples	--	--	--	--	50 µl
Leukotriene B_4 AChE Tracer	--	--	50 µl	50 µl	50 µl
Leukotriene B_4 Monoclonal Antibody	--	--	--	50 µl	50 µl
Incubate	Seal the plate and incubate plate overnight at 4°C				
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 90-120 minutes at room temperature on an orbital shaker protected from light				
Read	Read absorbance at 405-420 nm				

Table 6. Assay summary



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NOTES

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