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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Leukotriene B_4 Express ELISA Kit

Item No. 500003

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	Quantity/Size
400768	Leukotriene B_4 Express ELISA Monoclonal Antibody	1 vial/100 dtn
400767	Leukotriene B_4 Express AChE Tracer	1 vial/100 dtn
400766	Leukotriene B_4 Express ELISA Standard	1 vial/500 µl
400060	ELISA Buffer Concentrate (10X)	1 vial/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
400035	Polysorbate 20	1 vial/3 ml
400008/400009	Goat Anti-Mouse IgG-Coated Plate	1 plate
400012	96-Well Cover Sheet	1 ea
400050	Ellman's Reagent	3 vials/100 dtn
400040	ELISA Tracer Dye	1 ea
400042	ELISA Antiserum Dye	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. Adjustable pipettes and a repeating pipettor
3. An orbital microplate shaker
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

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 2 (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 Leukotriene B_4 Express ELISA Kit is a competitive assay that can be used for quantification of LTB_4 in plasma and other sample matrices. The assay has a range of 3.3-2,000 pg/ml, an average sensitivity (80%B/B_0) of 22 pg/ml, and a lower limit of detection of 3 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 Express AChE Tracer) for a limited number of LTB_4 monoclonal antibody binding sites. Because the concentration of the LTB_4 Express AChE Tracer is held constant while the concentration of free LTB_4 varies, the amount of LTB_4 Express AChE Tracer that is able to bind to the LTB_4 Express ELISA 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 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 Express AChE Tracer bound to the well, which is inversely proportional to the amount of free LTB_4 present in the well during the incubation, as described in the equation:

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

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

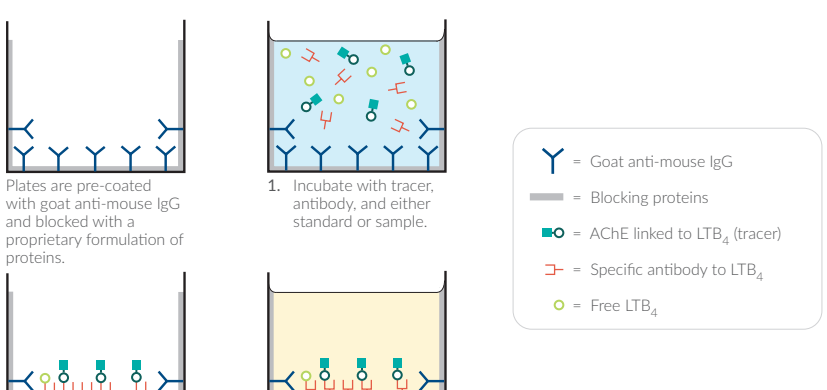


Figure 1. Schematic of the ELISA

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 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 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 midpoint (50% B/B_0) value of the tested molecule to the midpoint (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

Store all diluted buffers at 4°C; they will be stable for at least 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 Concentrate (10X)**

   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 Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 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 embarking on 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, which 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 and Serum

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate for plasma or without a coagulant for serum. Indomethacin should be added immediately after whole blood collection (sufficient to give a 10 µM final concentration). Indomethacin will prevent *ex vivo* formation of prostaglandins and thromboxanes, which have the potential to interfere with this assay. Centrifuge the whole blood at 1,500 x g at 4°C for 20 minutes and collect the plasma. The sample can be used immediately or stored at -80°C.

Plasma/Serum Purification

Plasma and serum can be purified using the Protein Precipitation Protocol below. Alternative protocols may be used based on the experimental requirements, sample type, and the end user's expertise.

Protein Precipitation Protocol

1. To precipitate proteins, add cold ethanol or acetone (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 supernatants to clean test tubes and evaporate the solvent under nitrogen.
4. Resuspend the samples in ELISA Buffer (1X) to its original volume, and use this for ELISA analysis. Samples can be concentrated in this step by using smaller volumes of buffer compared to the original sample volumes.

Sample Matrix Properties

Linearity

Human plasma samples were spiked with LTB_4, purified by protein precipitation as described in the **Plasma/Serum Purification** section (see page 14), serially diluted with ELISA Buffer (1X), and evaluated for linearity using the Leukotriene B_4 Express ELISA Kit. Results are shown in the table below.

Dilution Factor	Measured Concentration (pg/ml)	Linearity (%)
Heparin Plasma (Spiked with 2,000 pg/ml)		
8	2,847	100
16	2,568	90.2
32	2,824	99.2
Heparin Plasma (Spiked with 1,000 pg/ml)		
16	1,614	100
32	1,795	111
64	1,777	110
EDTA Plasma (Spiked with 1,000 pg/ml)		
12	2,154	100
24	1,972	91.6
48	2,035	94.5

Table 1. Linearity in human plasma

Spike and Recovery

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

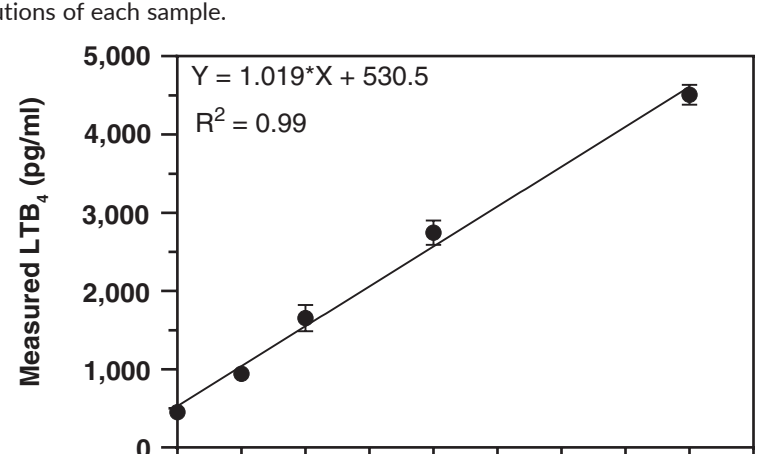


Figure 2. Spike and recovery of LTB_4 in human heparin plasma

Parallelism

To assess parallelism, human plasma samples were purified by protein precipitation as described in the **Plasma/Serum Purification** section (see page 14), serially diluted with ELISA Buffer (1X), and evaluated using the Leukotriene B_4 Express ELISA Kit. Measured concentrations were plotted as a function of the sample dilution. The results are shown below.

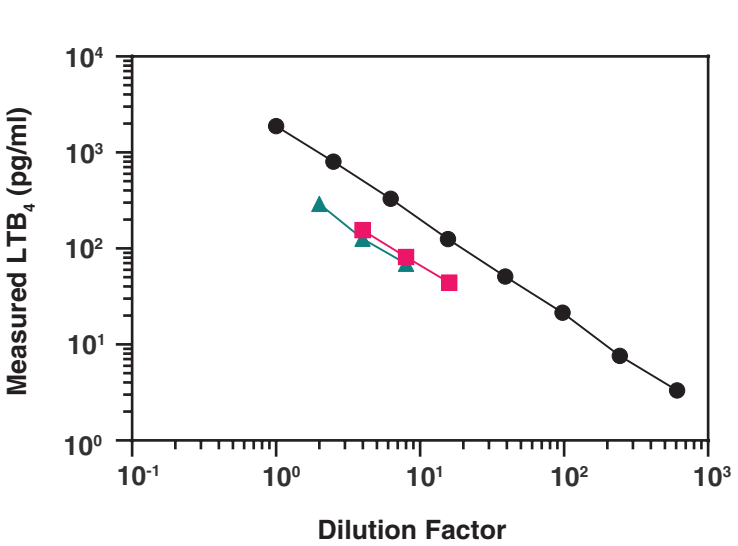


Figure 3. Parallelism of human plasma in the Leukotriene B_4 Express ELISA

Preparation of Assay-Specific Reagents

Leukotriene B_4 Express ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Leukotriene B_4 Express ELISA Standard (Item No. 400766). 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 solution at 4°C; it will be stable for approximately four weeks.

To prepare the standard for use in the ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 900 µl of ELISA Buffer (1X) to tube #1 and 600 µl of ELISA 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 400 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 400 µl from tube #2 and place into tube #3; mix thoroughly. Repeat this process for tubes #4-8. The diluted standards should not be stored for more than 24 hours.

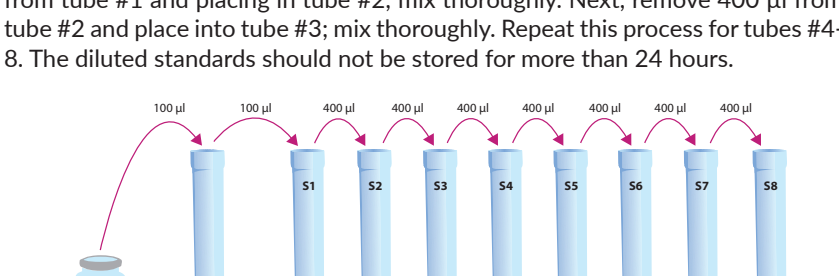


Figure 4. Preparation of the LTB_4 standards

Leukotriene B_4 Express AChE Tracer

Reconstitute the Leukotriene B_4 Express AChE Tracer (Item No. 400767) with 6 ml of ELISA Buffer (1X). Store the reconstituted LTB_4 Express 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). *NOTE: Do not store tracer with dye.*

Leukotriene B_4 Express ELISA Monoclonal Antibody

Reconstitute the Leukotriene B_4 Express ELISA Monoclonal Antibody (Item No. 400768) with 6 ml of ELISA Buffer (1X). Store the reconstituted LTB_4 Express ELISA Monoclonal Antibody at 4°C *(do not freeze!)*. It will be stable for 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). *NOTE: Do not store 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, assaying the samples in triplicate is recommended.

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 has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 24 for more details). It is suggested that the contents of each well be recorded 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
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. **ELISA Buffer (1X)**

   Add 100 µl of ELISA Buffer (1X) to NSB wells. Add 50 µl of ELISA Buffer (1X) to B_0 wells.

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

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

5. **Leukotriene B_4 Express ELISA Monoclonal Antibody**

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

Well	ELISA Buffer (1X)	Standard/ Sample	Tracer	Antibody
Blk	-	-	-	-
TA	-	-	5 µl (at devl. step)	-
NSB	100 µl	-	50 µl	-
B_0	50 µl	-	50 µl	50 µl
Std/Sample	-	50 µl	50 µl	50 µl

Table 2. Pipetting summary

Incubation of the Plate

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

Development of the Plate

1. Reconstitute Ellman's Reagent (Item No. 400050) immediately before use with 20 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 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. This assay typically develops (*i.e.*, B_0 wells ≥0.3 A.U. (Blk subtracted)) in 60-90 minutes.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings.*
3. Read the plate at a wavelength between 405 and 420 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 are in the range of 0.3-2.0 A.U. (Blk subtracted). If the absorbance of the wells exceeds 2.0 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 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 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* 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 using the equation obtained from the standard curve plot. *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, plot the absorbance values instead of %B/B_0 to calculate sample concentrations.*

Performance Characteristics

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 on page 27 to determine the values of your samples.

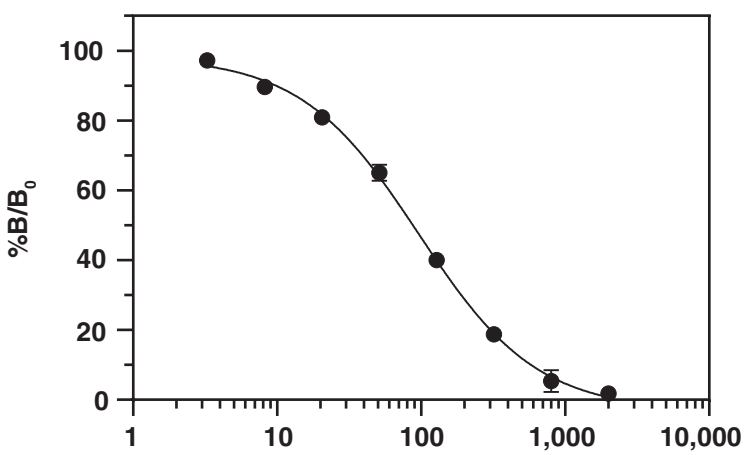
Absorbance at 414 nm (60 minutes)

LTB_4 (pg/ml) and Controls	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	$\%B/B_0$	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
TA	1.185	--	--	--	--
NSB	0.031	--	--	--	--
B_0	1.215	1.184	--	--	--
2,000	0.052	0.021	1.8	11.3	8.7
800	0.094	0.063	5.4	5.2	6.1
320	0.253	0.222	18.8	12.6	3.0
128	0.504	0.473	40.0	9.9	2.9
51.2	0.801	0.770	65.0	13.6	6.3
20.5	0.989	0.958	80.9	14.9	10.9
8.2	1.086	1.055	89.1	16.9	15.2
3.3	1.182	1.151	97.2	45.1**	50.3**

Table 3. 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 = 3.3-2,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 22.8 pg/ml
Mid-point (defined as 50% B/B_0) = 87.7 pg/ml
Lower Limit of Detection (LLOD) = 3.3 pg/ml

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

Figure 6. Typical standard curve

Precision:

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

Matrix Control (pg/ml)	%CV
1,046	6.1
273	7.1
69.8	9.7

Table 4. Intra-assay precision

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

Matrix Control (pg/ml)	%CV
1,239	1.3
242	7.8
49.6	5.3

Table 5. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity	Compound	Cross Reactivity
Leukotriene B_4	100%	Arachidonyl Ethanolamide	<0.01%
Leukotriene B_5*	166%	Prostaglandin $F_{2\alpha}$	<0.01%
20-hydroxy Leukotriene B_4	45.8%	12(S)-HETE	<0.01%
20-*epi* Leukotriene B_4	6.7%	Prostaglandin E_2	<0.01%
20-carboxy Leukotriene B_4	1.7%	5(S)-HETE	<0.01%
6-*trans* Leukotriene B_4	0.32%	(±)5(6)-DiHETE	<0.01%
6-*trans*-12-*epi* Leukotriene B_4	0.12%	Glutathione	<0.01%
Leukotriene D_4	0.02%	Leukotriene A_4	<0.01%
Resolvin D1	0.005%	Leukotriene C_4	<0.01%
15(S)-HETE	0.004%	Leukotriene E_4	<0.01%
Resolvin D2	0.002%	19(R)-hydroxy Prostaglandin B_2	<0.01%
15(R)-HETE	0.001%	Eicosapentaenoic Acid	<0.01%
Arachidonic Acid	<0.01%	5(R)-HETE	<0.01%

Table 6. Cross reactivity of the Leukotriene B_4 Express 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	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. 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	A. 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)	A. Standard has degraded B. There are dilution errors in the standard curve	A. Replace standards B. Re-run the standard curve with caution
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[16]
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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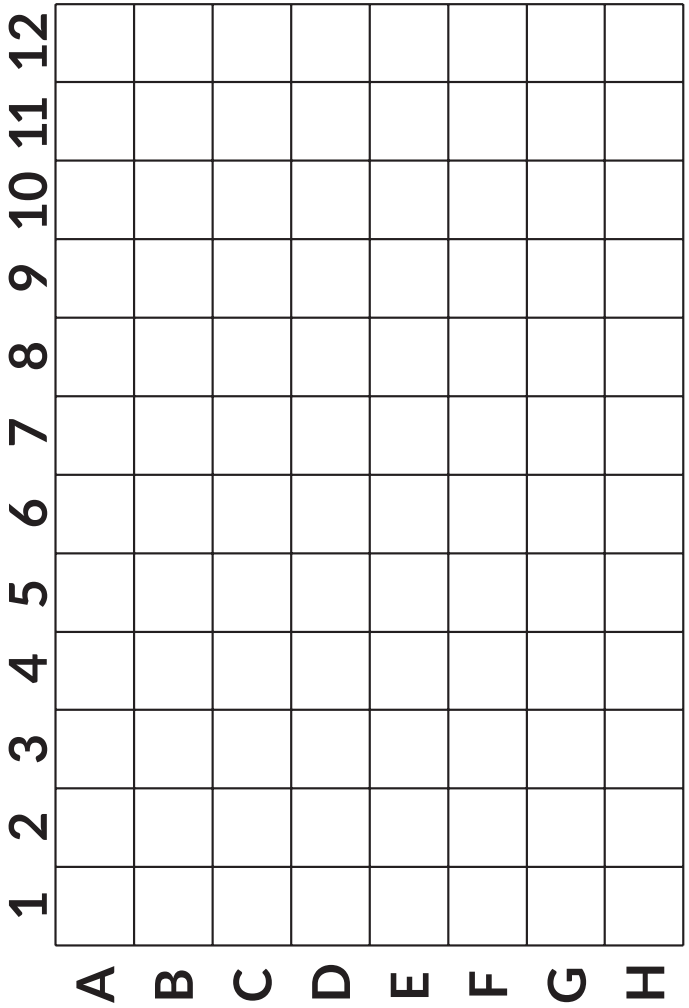

NOTES

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