



Leukotriene E₄ ELISA Kit

Item No. 501060

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TABLE OF CONTENTS

GENERAL INFORMATION	3	Materials Supplied
	4	Safety Data
	4	Precautions
	5	If You Have Problems
	5	Storage and Stability
	5	Materials Needed but Not Supplied
INTRODUCTION	6	Background
	6	About This Assay
	8	Principle Of This Assay
	10	Definition of Key Terms
PRE-ASSAY PREPARATION	12	Buffer Preparation
	13	Sample Preparation
	14	Sample Matrix Properties
ASSAY PROTOCOL	15	Preparation of Assay-Specific Reagents
	18	Plate Set Up
	19	Performing the Assay
ANALYSIS	23	Calculations
	26	Performance Characteristics
RESOURCES	31	Troubleshooting
	32	References
	33	Plate Template
	34	Notes
	35	Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
401062	Leukotriene E ₄ ELISA Antiserum	1 vial/100 dtn	1 vial/500 dtn
401060	Leukotriene E ₄ AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
420414	Leukotriene E 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
400004/400006	Mouse Anti-Rabbit 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.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Leukotriene E₄ ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

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

If You Have Problems

Technical Service Contact Information

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

Email: techserv@caymanchem.com

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

Storage and Stability

This kit will perform as specified if stored as directed at -80°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)

Background

Leukotriene E₄ (LTE₄) is a product of the 5-lipoxygenase (5-LO) pathway in activated mast cells, eosinophils, and monocytes, and is one of the constituents of slow-reacting substance of anaphylaxis (SRS-A).^{1,2} LTA₄, the primary 5-LO metabolite, is converted to LTC₄ and sequentially to LTD₄ and LTE₄ in the host cell, or by transcellular metabolism in erythrocytes, platelets, or neutrophils (see Figure 1, page 7).¹ LTE₄ is produced by the action of dipeptidase on LTD₄, leaving only the cysteinyl group still attached to the fatty acid backbone.³ It is considerably less active than LTC₄ in the biological activities characteristic of cysteinyl leukotrienes (CysLTs), including bronchoconstriction and vasoconstriction.^{3,4} LTE₄ has been found in urine, bile, and plasma, though it has a short plasma half-life.^{5,6} Urine levels of LTE₄ are increased in patients with asthma during episodes of bronchoconstriction.⁷

About This Assay

Cayman's LTE₄ ELISA Kit is a competitive assay that can be used for the quantification of LTE₄ in urine without purification. The assay has a range of 7.8-1,000 pg/ml with a sensitivity (80% B/B₀) of approximately 25 pg/ml and a lower limit of detection (LLOD) of 6.9 pg/ml.

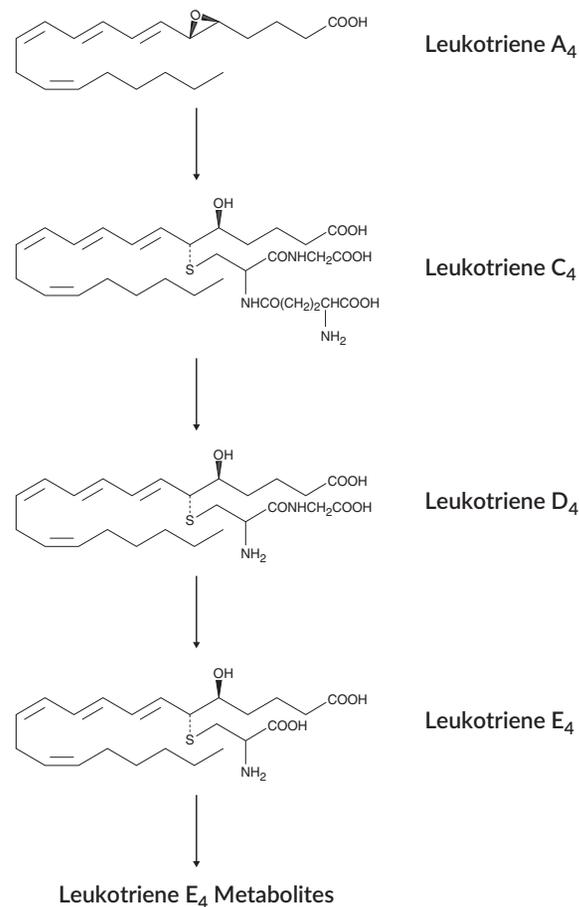


Figure 1. The formation of CysLTs

Principle Of This Assay

This assay is based on the competition between free LTE_4 and an LTE_4 -acetylcholinesterase (AChE) conjugate (LTE_4 -AChE Tracer) for a limited amount of LTE_4 antibody binding sites. Because the concentration of the LTE_4 -AChE Tracer is held constant while the concentration of free LTE_4 varies, the amount of LTE_4 -AChE Tracer that is able to bind to the LTE_4 polyclonal antibody will be inversely proportional to the concentration of free LTE_4 in the well. This antibody- LTE_4 complex binds to mouse anti-rabbit 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 LTE_4 -AChE Tracer bound to the well, which is inversely proportional to the amount of free LTE_4 present in the well during the incubation, as described in the equation:

$$\text{Absorbance} \propto [\text{bound } \text{LTE}_4\text{-AChE tracer}] \propto 1/[\text{LTE}_4]$$

A schematic of this process is shown in Figure 2, on page 9.

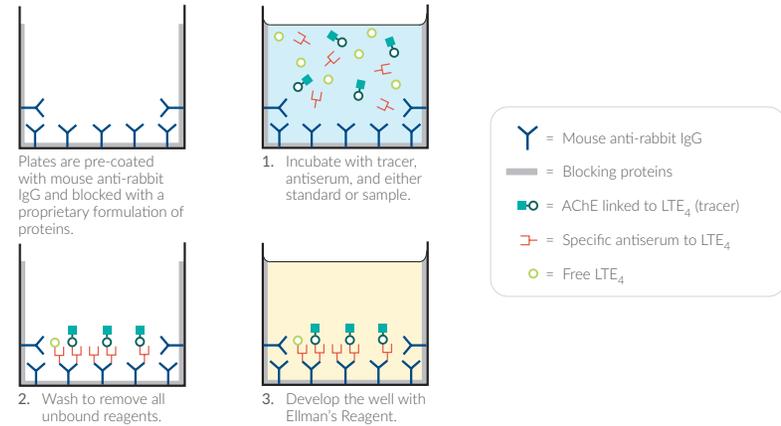


Figure 2. Schematic of the ELISA

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 non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the LTE₄-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.

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

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

Standard Curve: a plot of the %B/B₀ 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/B₀) value of the tested molecule to the mid-point (50% B/B₀) value of the primary analyte when each is measured in assay buffer using the following formula:

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

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.

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

Testing for Interference

This assay has been validated in urine. Other sample types should be tested for interference to evaluate the need for sample purification before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions within the linear portion of the standard curve. If two different dilutions of the same sample show good correlation (differ by 20% or less) in the final calculated LTE₄ concentration, sample purification is not required. If you do not see good correlation of the different dilutions, purification is advised. Purification methods will need to be determined by the end user and tested for compatibility in the assay.

Urine

Urine samples should be assayed immediately or stored at -20°C immediately after collection. Interference in urine is infrequent. Dilute urine sample in ELISA Buffer (1X) to fall within the range of the standard curve. 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.

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

Sample Matrix Properties

Spike and Recovery

Human urine samples were spiked with LTE₄, diluted with ELISA Buffer (1X) as described in the Sample Preparation section and analyzed using the LTE₄ ELISA Kit. Error bars represent standard deviations obtained from multiple dilutions of each sample.

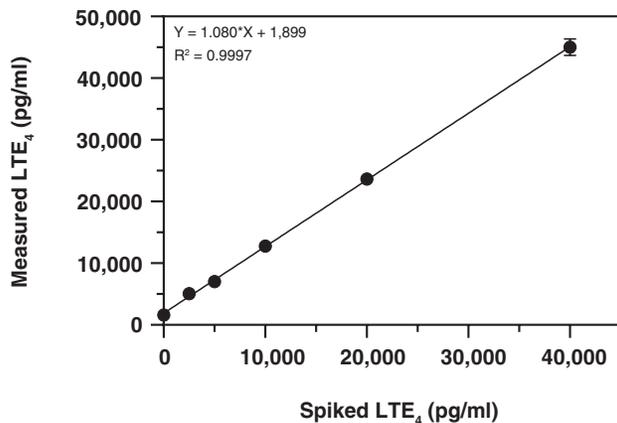


Figure 3. Spike and recovery of LTE₄ in human urine

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

LTE₄ ELISA Standard

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with LTE₄ ELISA Standard (Item No. 420414) several times. 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 10 ng/ml.

NOTE: If assaying culture medium samples, culture medium should be used in place of ELISA Buffer (1X) for dilution of the standard curve and samples.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number 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 (10 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 be used within 24 hours.

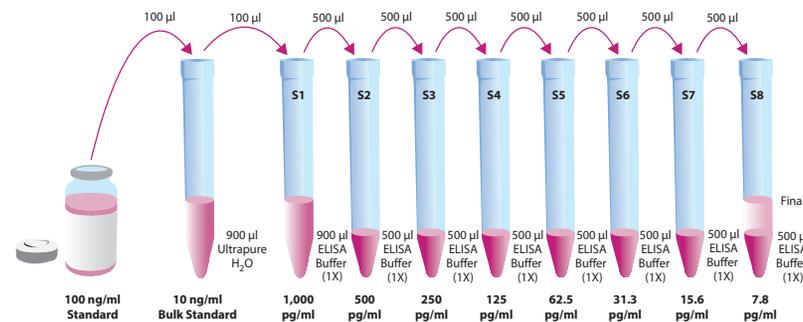


Figure 4. Preparation of the LTE₄ standards

LTE₄-AChE Tracer

Reconstitute the LTE₄-AChE Tracer as follows:

100 dtn LTE₄-AChE Tracer (96-well kit; Item No. 401060): Reconstitute with 6 ml ELISA Buffer (1X) and transfer to a polypropylene tube or vial.

OR

500 dtn LTE₄-AChE Tracer (480-well kit; Item No. 401060): Reconstitute with 1 ml ELISA Buffer (1X) and transfer to a polypropylene container that will hold at least 30 ml. Add 29 ml ELISA Buffer (1X).

Store the reconstituted LTE₄-AChE Tracer at 4°C (*do not freeze!*) and use within 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).

LTE₄ ELISA Antiserum

Reconstitute the LTE₄ ELISA Antiserum as follows:

100 dtn LTE₄ ELISA Antiserum (96-well kit; Item No. 401062): Reconstitute with 6 ml ELISA Buffer (1X) and transfer to a polypropylene tube or vial.

OR

500 dtn LTE₄ ELISA Antiserum (480-well kit; Item No. 401062): Reconstitute with 1 ml ELISA Buffer (1X) and transfer to a polypropylene container that will hold at least 30 ml. Add 29 ml ELISA Buffer (1X).

Store the reconstituted LTE₄ Antiserum at 4°C (*do not freeze!*). It will be stable for at least four weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antiserum, if desired, to aid in visualization of antiserum-containing wells. Add the dye to the reconstituted antiserum at a final dilution of 1:100 (add 60 µl of dye to 6 ml antiserum or add 300 µl of dye to 30 ml of antiserum).

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 B₀, 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 23, for more details). We suggest 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
B₀ = Maximum Binding wells
S1-S8 = Standard wells
1-24 = Sample wells

Figure 5. Sample plate format

Performing the Assay

Equilibrate all reagents at room temperature prior to addition to the plate.

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.
- Equilibrate all reagents at room temperature prior to addition to the plate.

Addition of the Reagents

1. ELISA Buffer

Add 150 µl ELISA Buffer (1X) to NSB wells. Add 100 µl ELISA Buffer (1X) to B₀ wells.

2. LTE₄ ELISA Standard

Add 100 µl from tube #8 to both of the lowest standard wells (S8). Add 100 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 100 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. LTE₄-AChE Tracer

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

5. LTE₄ ELISA Antiserum

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

Well	ELISA Buffer	Standard/ Sample	Tracer	Antiserum
Blk	-	-	-	-
TA	-	-	5 µl (at devl. step)	-
NSB	150 µl	-	50 µl	-
B ₀	100 µl	-	50 µl	50 µl
Std/Sample	-	100 µl	50 µl	50 µl

Table 1. Pipetting summary

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate 18 hours 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 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.*, B₀ 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 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 405-420 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, 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/analysisTools/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/B₀ for standards S1-S8 versus LTE₄ concentration using linear (y) and log (x) axes and perform a four-parameter logistic fit.

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

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

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

Determine the Sample Concentration

Calculate the %B/B₀ (or B/B₀) value for each sample. Determine the concentration of each sample by identifying the %B/B₀ on the standard curve and reading the corresponding values on the x-axis. *NOTE: Remember to account for any concentration or dilution of the original sample prior to its addition to the well.* Samples with %B/B₀ values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample could be an indicator of interference, which could be eliminated by purification.

NOTE: If there is an error in the B₀ wells, plot the absorbance values instead of %B/B₀ to calculate sample concentrations.

Performance Characteristics

Representative Data

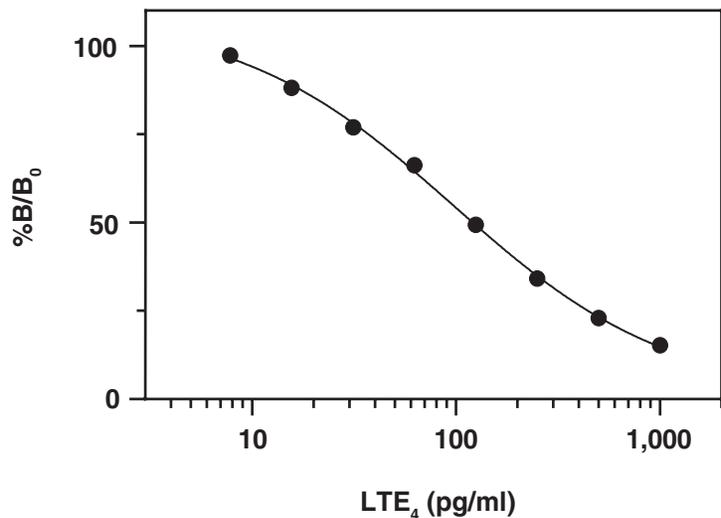
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples.

Absorbance at 414 nm (90 minutes)

LTE ₄ Standards (pg/ml) and Controls	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	%B/B ₀	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
NSB	0.004	--	--	--	--
B ₀	0.647	0.643	--	--	--
TA	0.326	--	--	--	--
1,000	0.099	0.095	16.2	9.4	7.5
500	0.146	0.142	24.2	9.5	12.3
250	0.210	0.206	35.1	10.2	9.7
125	0.300	0.296	50.3	13.2	13.4
62.5	0.400	0.396	67.2	14.5	7.2
31.3	0.452	0.448	76.1	15.4	10.3
15.6	0.509	0.505	85.7	19.9	17.0
7.8	0.569	0.565	96.1	20.4	20.4

Table 2. Typical results

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



Assay Range = 7.81-1,000 pg/ml
Sensitivity (defined as 80% B/B₀) = 25 pg/ml
Mid-point (defined as 50% B/B₀) = 127 pg/ml
Lower Limit of Detection (LLOD) = 6.9 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 urine controls in a single assay.

Matrix Control (pg/ml)	%CV
1,276.00	15.4
254.25	10.4
198.99	4.8

Table 3. Intra-assay precision

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

Matrix Control (pg/ml)	%CV
1,335.75	14
253.25	12.6
179.26	11.1

Table 4. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity
LTE ₄	100%
LTB ₄	<0.1%
Arachidonic Acid	<0.01%
LTB ₅	<0.01%
LTC ₄	<0.01%
LTD ₄	<0.01%
11- <i>trans</i> LTE ₄	<0.01%
N-acetyl LTE ₄	<0.01%
tetranor-PGEM	<0.01%
tetranor-PGFM	<0.01%
Docosahexaenoic Acid	<0.01%
Eicosapentaenoic Acid	<0.01%

Table 5. Cross reactivity of the LTE₄ ELISA

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 antiserum
Very low B ₀	A. Trace organic contaminants in the water source B. Plate requires additional development time C. Dilution error in preparing reagents
Low sensitivity (shift in dose-response curve)	A. Standard is degraded B. Dilution error in preparing standards
Analyses of two dilutions of a biological sample do not agree (<i>i.e.</i> , 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 of the samepl 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

References

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