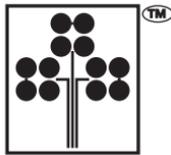


Leukotriene E₄ EIA Kit

Item No. 520411



ACE

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
420412	Leukotriene E ₄ EIA Antiserum	1 vial/100 dtn	1 vial/500 dtn
420410	Leukotriene E ₄ AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
420414	Leukotriene E ₄ EIA Standard	1 vial	1 vial
400060	EIA 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
400005/400007	Mouse Anti-Rabbit IgG Coated Plate	1 plate	5 plates
400012	96-Well Cover Sheet	1 cover	5 covers
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
400040	EIA Tracer Dye	1 vial	1 vial
400042	EIA Antiserum Dye	1 vial	1 vial

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

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 ACE™ EIA Kits. This kit may not perform as described if any reagent or procedure is replaced or modified.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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. Adjustable pipettes and a repeat pipettor.
3. A source of 'UltraPure' water. Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 13).

INTRODUCTION

Background

Leukotriene E₄ (LTE₄) is a product of the 5-lipoxygenase (5-LO) pathway in activated mast cells, eosinophils, and monocytes.¹ 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). This metabolism is rapid and complete, in that plasma levels of LTC₄ are virtually undetectable. Exogenously administered LTC₄ is recovered in the urine as LTE₄ (5-13%) and two prominent oxidized metabolites resulting from several cycles of β-oxidation.² The plasma half-life of LTE₄ is about 7 minutes. Plasma LTE₄ levels are likewise <2 pg/ml as a consequence of the low rate of production and rapid elimination.³

Normal human urine contains low but detectable amounts of LTE₄, ranging from 10-60 pg/ml.⁴ Asthmatic patients in an acute episode of bronchoconstriction may have elevations of urinary LTE₄ to several hundred pg/ml, but their baseline LTE₄ levels are not consistently abnormal. Methods for the rapid isolation and detection of LTE₄ from human urine have been developed.^{5,6}

About This Assay

Cayman's LTE₄ EIA Kit is a competitive assay that can be used for quantification of LTE₄ in urine, plasma, serum, whole blood, as well as other heterogeneous mixtures such as lavage fluids and aspirates and other sample matrices. The EIA typically displays an IC₅₀ (50% B/B₀) of approximately 100 pg/ml and a detection limit (80% B/B₀) of approximately 25 pg/ml.

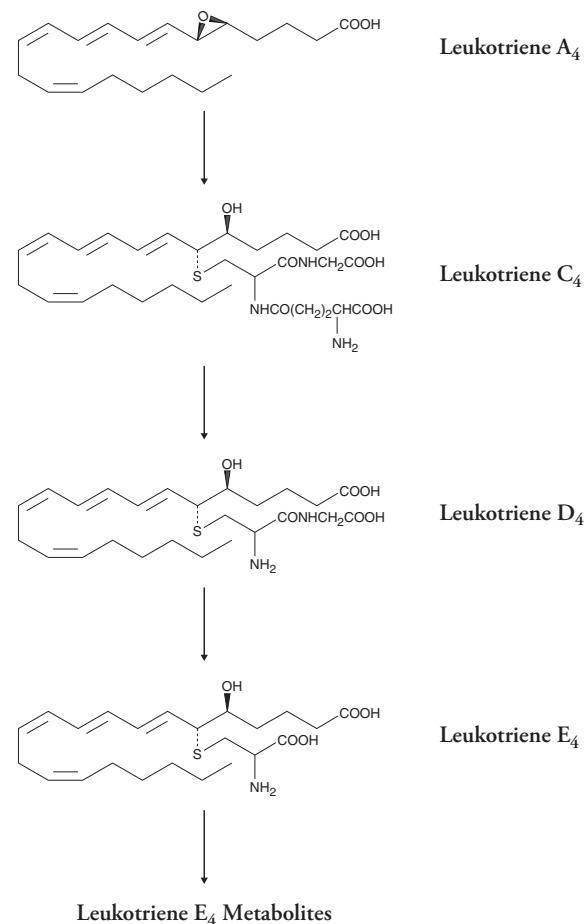


Figure 1. Metabolism of LTE₄

Description of ACE™ Competitive EIAs^{7,8}

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

$$\text{Absorbance} \propto [\text{Bound } \text{LTE}_4 \text{ Tracer}] \propto 1/[\text{LTE}_4]$$

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

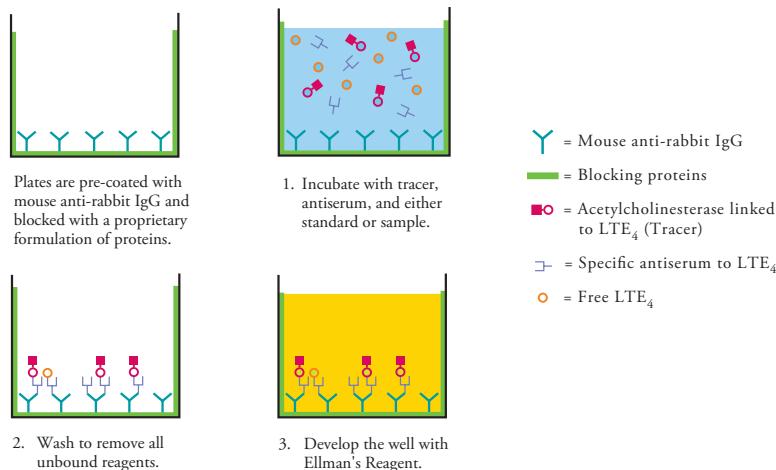


Figure 2. Schematic of the ACE™ EIA

Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *Electrophorus electricus*, contains an avid acetylcholinesterase (AChE) capable of massive catalytic turnover during the generation of its electrochemical discharges. The electric eel AChE has a clover leaf-shaped tertiary structure consisting of a triad of tetramers attached to a collagen-like structural fibril. This stable enzyme is capable of high turnover ($64,000 \text{ s}^{-1}$) for the hydrolysis of acetylthiocholine.

A molecule of the analyte covalently attached to a molecule of AChE serves as the tracer in ACE™ enzyme immunoassays. Quantification of the tracer is achieved by measuring its AChE activity with Ellman's Reagent. This reagent consists of acetylthiocholine and 5,5'-dithio-*bis*-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by AChE produces thiocholine (see Figure 3, on page 10). The non-enzymatic reaction of thiocholine with 5,5'-dithio-*bis*-(2-nitrobenzoic acid) produces 5-thio-2-nitrobenzoic acid, which has a strong absorbance at 412 nm ($\epsilon = 13,600$).

AChE has several advantages over other enzymes commonly used for enzyme immunoassays. Unlike horseradish peroxidase, AChE does not self-inactivate during turnover. This property of AChE also allows re-development of the assay if it is accidentally splashed or spilled. In addition, the enzyme is highly stable under the assay conditions, has a wide pH range (pH 5-10), and is not inhibited by common buffer salts or preservatives. Since AChE is stable during the development step, it is unnecessary to use a 'stop' reagent, and the plate may be read whenever it is convenient.

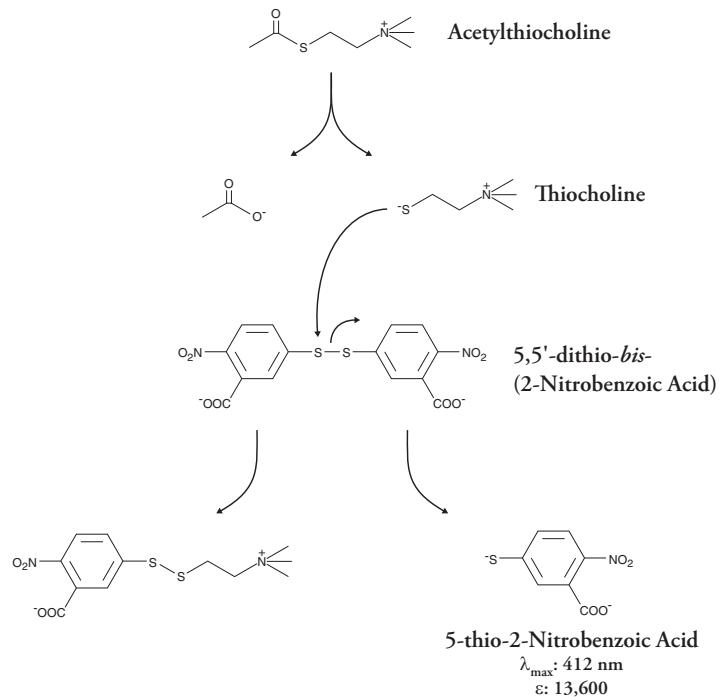


Figure 3. Reaction catalyzed by acetylcholinesterase

Definition of Key Terms

Blank: background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells.

Total Activity: total enzymatic activity of the AChE-linked tracer. This is analogous to the specific activity of a radioactive 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 particular sample or standard well to that of the maximum binding (B_0) well.

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, where one dtn is the amount of reagent used per well.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may be purchased from Cayman (Item No. 400000).

Buffer Preparation

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

1. EIA Buffer Preparation

Dilute the contents of one vial of EIA 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. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. Wash Buffer Preparation

5 ml vial Wash Buffer Concentration (400X) (96-well kit; Item No. 400062):

Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20 (Item No. 400035).

OR

12.5 ml vial Wash Buffer Concentration (400X) (480-well kit; Item No. 400062):

Dilute to a total volume of 5 liters with UltraPure water and add 2.5 ml of Polysorbate 20 (Item No. 400035).

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

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

Urine, plasma, serum, whole blood, as well as other heterogeneous mixtures such as lavage fluids and aspirates often contain contaminants which can interfere in the assay. The presence of rabbit IgG in the sample will interfere in the assay. It is best to check for interference before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to at least two different dilutions between approximately 25 and 1,000 pg/ml (*i.e.*, between 20-80% B/B₀). If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated LTE₄ concentration, purification is not required. If you do not see good correlation of serial dilutions, purification is advised. The Purification Protocol, on page 15, is one such method. *NOTE: We recommend using Cayman's Cysteinyl Leukotriene Affinity Sorbent (Item No. 400396) or Cayman's Cysteinyl Leukotriene Affinity Columns (Item Nos. 400068 and 400069) for purification of LTE₄ from biological samples prior to EIA analysis.*

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.

Lavage Fluids and Aspirates

CysLTs can accumulate to relatively high concentrations in the effusion fluids associated with inflammation (*e.g.*, ascites fluid, synovial fluid, pleural effusion, pericardial or cerebral intraventricular aspirates). Since LT metabolism is incomplete in these circumstances, substantial amounts of LTC₄, LTD₄, and LTE₄ may be present (*e.g.*, bronchoalveolar lavage fluid from asthmatic subjects may contain 700-1,000 pg/ml CysLTs comprised mainly of LTC₄ and LTD₄).⁹ Consequently, analysis of these fluids is the optimal application of the Cysteinyl Leukotriene EIA Kit (Item No. 500390) or Cysteinyl Leukotriene Express EIA Kit (Item No. 10009291). *NOTE: The complex nature of these samples makes purification mandatory in order to achieve accurate results.*^{9,10}

Urine

CysLTs are excreted in urine as intact LTE₄ (~9-12%) and LTE₄ metabolites. Levels of intact LTE₄ in human urine are 50-80 pg/mg creatinine (about 10-50 pg/ml).⁹ Since LTC₄ and LTD₄ are virtually absent from urine, CysLT measurement in urine samples is often best accomplished by measuring LTE₄ using this kit. Unpurified urine samples show a linear but elevated concentration of CysLTs. Therefore, to increase the accuracy of the data generated, urine samples should be purified prior to assay.¹¹

Culture Medium Samples

Cultured cells synthesizing LTC₄ will generally release it into the medium where it will accumulate without further metabolism. Thus, samples of this type are best analyzed by the measurement of LTC₄ specifically (LTC₄ EIA Kit, Item No. 520211).

Plasma

Metabolism of LTC₄ in plasma is rapid and complete, after which the metabolites are eliminated and recovered in the urine as LTE₄. Plasma levels of LTE₄ are therefore very low (<2 pg/ml) (see **Biochemistry of Leukotriene E₄** for additional details). Plasma samples should be collected in vacutainers containing sodium heparin, EDTA, or sodium citrate. Due to the low levels of LTE₄ in plasma, as well as it being a complex matrix that contains many substances that can interfere with this assay, purification is necessary prior to performing the assay.

Sample Purification

Cayman Chemical highly recommends the use of our affinity purification reagents for the rapid and easy purification of cysteinyl leukotrienes from biological samples (Cysteinyl Leukotriene Affinity Sorbent - Item No. 400396; Cysteinyl Leukotriene Affinity Columns - Item Nos. 400068 and 400069). These reagents are easier to use and provide higher purity with better recovery than solid phase extraction (SPE) chromatography. In particular, urinary LTE₄ cannot be sufficiently purified using SPE (C-18). Impurities in urine co-elute with LTE₄ from SPE Cartridges (C-18) and interfere in the EIA measurement. Therefore, the options for purification of urinary LTE₄ are to either use the affinity reagents or use SPE (C-18) followed by HPLC/TLC. The protocol for affinity purification accompanies those reagents when purchased.

SPE (C-18) Purification Protocol

The following protocol is a suggestion only. You may choose a different protocol based on your own requirements, sample type, and expertise. If desired, recovery may be tracked by spiking samples with tritium-labeled LTE₄ (³H]-LTE₄) and follow the spiked-sample recovery calculations in the **Analysis** section on page 26. Otherwise, omit steps 2 and 9.

Materials Needed

1. Tritium-labeled LTE₄ (optional)
2. 1 M acetate buffer (pH 4), deionized water, and methanol
3. C-18 solid phase extraction (SPE) columns (Item No. 400020)

Protocol

1. Aliquot a known amount of each sample into a clean test tube (500 µl is recommended). If your 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. Add 10,000 cpm of tritium-labeled LTE₄ (³H]-LTE₄). Use a high specific activity tracer to minimize the amount of radioactive LTE₄ as the EIA will be able to detect the added LTE₄.
3. *Precipitation of proteins using ethanol is optional and may not be needed if samples are clean enough to flow through the SPE Cartridge (C-18). Body fluids such as plasma and urine can typically be applied directly to the SPE Cartridge (C-18) after the acidification step (step 4) below.* To precipitate proteins, add methanol (approximately four times the sample volume) to each tube. Vortex to mix thoroughly. Incubate samples at 4°C for five minutes, then centrifuge a 3,000 x g for 10 minutes to remove precipitated proteins. Transfer the supernatant to a clean test tube. Evaporate the methanol either by vacuum centrifugation or under a gentle stream of nitrogen.

4. Adjust the pH to ~4 by the addition of 1 M acetate buffer (citrate buffer or dilute HCl). To avoid having to measure the pH of each individual sample, adjust the pH of an equivalent volume of sample matrix to pH 4 using 1 M acetate buffer. Add this volume of buffer to each sample. *NOTE: For samples of different volumes, the amount of buffer should be adjusted to maintain this ratio of buffer to sample.* 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.
5. Activate a 6 ml SPE Cartridge (C-18) (Item No. 400020) by rinsing with 5 ml methanol and followed by 5 ml UltraPure water. Do not allow the SPE Cartridge (C-18) to dry.
6. Pass the sample through the SPE Cartridge (C-18). Rinse the cartridge with 5 ml UltraPure water. Discard the wash. Elute the LTE₄ with 5 ml methanol. 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 methanol to dryness either by vacuum centrifugation or by evaporation under a stream of dry nitrogen. It is imperative that all of the organic solvent be removed as even trace quantities will adversely affect the EIA.
8. Add 500 µl of EIA Buffer and vortex. Use this for EIA analysis. It is common for an insoluble precipitate to remain after the addition of EIA Buffer; this will not affect the assay.

*If it is necessary to stop during this purification, samples may be stored in the methanol solution at -80°C.
9. Use 50 µl of the resuspended sample for scintillation counting.

Preparation of Assay-Specific Reagents

Leukotriene E₄ EIA Standard

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 100 µl of the LTE₄ EIA Standard (Item No. 420414) 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 that have not been diluted with EIA Buffer, culture medium should be used in place of EIA Buffer for dilution of the standard curve.

To prepare the standard for use in EIA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl EIA Buffer to tube #1 and 500 µl EIA Buffer 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 not be stored for more than 24 hours.

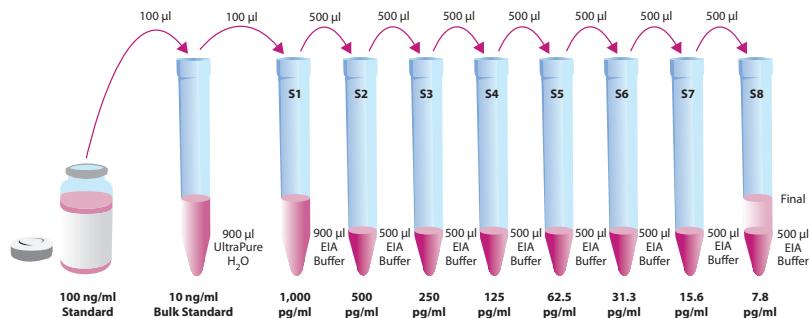


Figure 4. Preparation of the LTE₄ standards

Leukotriene E₄ AChE Tracer

Reconstitute the LTE₄ AChE Tracer as follows:

100 dtn LTE₄ AChE Tracer (96-well kit; Item No. 420410): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn LTE₄ AChE Tracer (480-well kit; Item No. 420410): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted LTE₄ AChE Tracer at 4°C (*do not freeze!*) and use within four weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

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

Leukotriene E₄ EIA Antiserum

Reconstitute the LTE₄ EIA Antiserum as follows:

100 dtn LTE₄ EIA Antiserum (96-well kit; Item No. 420412): Reconstitute with 6 ml EIA Buffer.

OR

500 dtn LTE₄ EIA Antiserum (480-well kit; Item No. 420412): Reconstitute with 30 ml EIA Buffer.

Store the reconstituted LTE₄ EIA Antiserum at 4°C. It will be stable for at least four weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

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

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you 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 blanks (Blk), two non-specific binding wells (NSB), two maximum binding wells (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 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 24, for more details). We suggest you record the contents of each well on the template sheet provided (see page 35).

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

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

Figure 5. Sample plate format

Performing the Assay

Pipetting Hints

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

Addition of the Reagents

1. EIA Buffer

Add 100 µl EIA Buffer to Non-Specific Binding (NSB) wells. Add 50 µl EIA Buffer to Maximum Binding (B₀) wells. If culture medium was used to dilute the standard curve, substitute 50 µl of culture medium for EIA Buffer in the NSB and B₀ wells (*i.e.*, add 50 µl culture medium to NSB and B₀ wells and 50 µl EIA Buffer to NSB wells).

2. Leukotriene E₄ EIA 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 E₄ AChE Tracer

Add 50 µl to each well *except* the Total Activity (TA) and the Blank (Blk) wells.

5. Leukotriene E₄ EIA Antiserum

Add 50 µl to each well *except* the Total Activity (TA), the Non-Specific Binding (NSB), and the Blank (Blk) wells.

Well	EIA Buffer	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 1. Pipetting summary

Incubation of the Plate

Cover each plate with plastic film (Item No. 400012) and incubate 18 hours at room temperature.

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 Wash Buffer.
3. Add 200 µl of Ellman's Reagent to each well.
4. Add 5 µl of tracer to the Total Activity wells.
5. Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (*i.e.*, B₀ wells ≥0.3 A.U. (blank subtracted)) in 60-90 minutes.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings. If Ellman's Reagent is present on the cover, use a pipette to transfer the Ellman's Reagent into the well. If too much Ellman's Reagent has splashed on the cover to easily redistribute back into the wells, wash the plate three times with wash buffer and repeat the development with fresh Ellman's Reagent.*
3. Read the plate at a wavelength between 405 and 420 nm. The absorbance may be checked periodically until the B₀ wells have reached a minimum of 0.3 A.U. (blank subtracted). The plate should be read when the absorbance of the B₀ wells are in the range of 0.3-1.0 A.U. (blank subtracted). If the absorbance of the wells exceeds 1.5, 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/B₀ versus log concentration using a four-parameter logistic fit or as logit B/B₀ 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/ia) 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 B₀ wells.
3. Subtract the NSB average from the B₀ average. This is the corrected B₀ or corrected maximum binding.
4. Calculate the B/B₀ (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 B₀ (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B₀ for a logistic four-parameter fit, multiply these values by 100.)

*NOTE: The total activity (TA) values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool; the corrected B₀ divided by the actual TA (10X measured absorbance) will give the % Bound. This value should closely approximate the % Bound that can be calculated from the **Sample Data** (see page 27). Erratic absorbance values and a low (or no) % Bound could indicate the presence of organic solvents in the buffer or other technical problems (see page 31 for **Troubleshooting**).*

Plot the Standard Curve

Plot %B/B₀ for standards S1-S8 versus LTE₄ concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

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

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

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

Determine the Sample Concentration

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

Spiked-Sample Recovery Calculation

$$\text{Recovery Factor} = \frac{10 \times \text{cpm of sample}}{[^3\text{H}]\text{-LTE}_4 \text{ added to sample (cpm)}}$$

LTE₄ (pg) in purified sample =

$$\left[\frac{\text{Value from EIA (pg/ml)}}{\text{Recovery Factor}} \right] \times 0.5 \text{ ml} - \text{added } [^3\text{H}]\text{-LTE}_4 \text{ (pg)}$$

Total LTE₄ in sample (pg/ml) =

$$\frac{\text{LTE}_4 \text{ (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}}$$

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 below to determine the values of your samples. Your results could differ substantially.

	Raw Data		Average	Corrected
Total Activity	2.790	2.700	2.745	
NSB	0.003	0.003	0.003	
B₀	0.378	0.389		
	0.379	0.371	0.379	0.376

Dose (pg/ml)	Raw Data		Corrected		%B/B ₀	
1,000	0.072	0.069	0.069	0.066	18.2	17.4
500	0.094	0.098	0.091	0.095	24.1	25.1
250	0.131	0.134	0.128	0.131	33.9	34.7
125	0.165	0.166	0.162	0.163	43	43.2
62.5	0.209	0.206	0.206	0.203	54.7	53.9
31.3	0.249	0.261	0.246	0.258	65.3	68.5
15.6	0.299	0.304	0.296	0.301	78.6	80
7.8	0.322	0.347	0.319	0.344	84.8	91.4

Table 2. Typical results

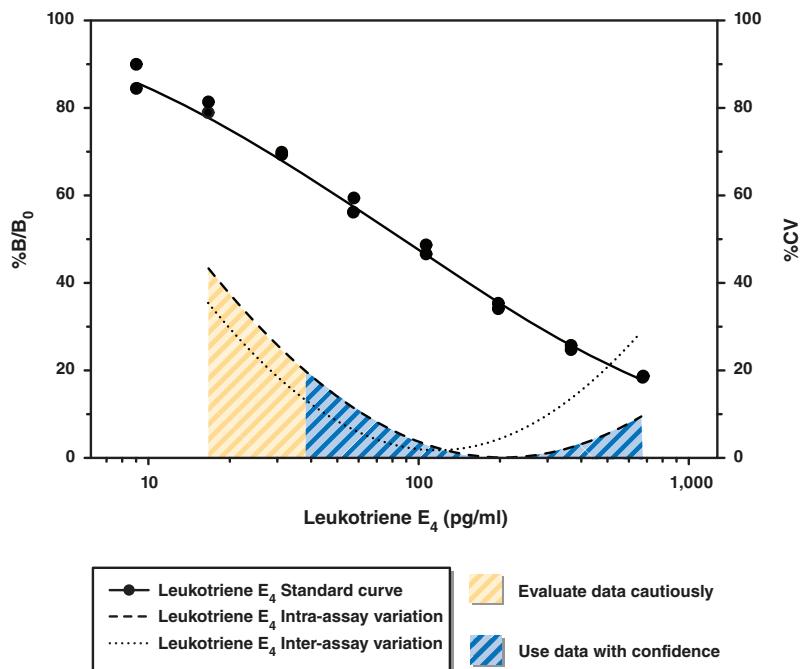


Figure 6. Typical standard curve

Precision:

The intra- and inter-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 28 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
1,000	4.5	28.3
500	6.4	9.2
250	6.5	10.0
125	7.1	7.9
62.5	8.0	9.0
31.3	11.0	10.0
15.6	53.3	25.9
7.8	146.6	68.8

Table 3. Intra- and inter-assay variation

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

Specificity:

Compound	Cross Reactivity
Leukotriene E ₄	100%
Leukotriene E ₅	100%
11- <i>trans</i> Leukotriene E ₄	66.3%
N-acetyl Leukotriene E ₄	20%
Leukotriene C ₄	10%
Leukotriene D ₄	7%
Leukotriene C ₅	2%
Arachidonic Acid	<0.01%
Leukotriene B ₄	<0.01%
Leukotriene B ₅	<0.01%
Leukotriene D ₅	<0.01%
tetranor-PGEM	<0.01%
tetranor-PGFM	<0.01%

Table 4. Specificity of the LTE₄ EIA Antiserum

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of UltraPure water
High NSB (>0.035)	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)	Standard is degraded	Replace standard
Analyses of two dilutions of a biological sample do not agree (<i>i.e.</i> , more than 20% difference)	Interfering substances are present	Purify sample prior to analysis by EIA ¹¹
Only Total Activity (TA) wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of UltraPure water

Additional Reading

Go to www.caymanchem.com/520411/references for a list of publications citing the use of Cayman's LTE₄ EIA Kit.

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Related Products

Creatinine (serum) Assay Kit - Item No. 700460
Cysteinyl Leukotriene Affinity Purification Kit (4 ml) - Item No. 10010392
Cysteinyl Leukotriene Affinity Purification Kit (20 ml) - Item No. 520503
Cysteinyl Leukotriene Affinity Sorbent - Item No. 400396
Cysteinyl Leukotriene EIA Kit - Item No. 500390
Cysteinyl Leukotriene Express EIA Kit - Item No. 10009291
Histamine EIA Kit - Item No. 589651
Leukotriene C₄ EIA Kit - Item No. 520211
SPE Cartridges (C-18) - Item No. 400020
UltraPure Water - Item No. 400000

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NOTES

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