



## Leukotriene C<sub>4</sub> ELISA Kit

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Item No. 501070

[www.caymanchem.com](http://www.caymanchem.com)

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## GENERAL INFORMATION

### Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
401072	Leukotriene C <sub>4</sub> ELISA Antiserum	1 vial/100 dtn	1 vial/500 dtn
401070	Leukotriene C <sub>4</sub> AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
420214	Leukotriene C <sub>4</sub> ELISA Standard	1 vial	1 vial
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 cover	5 covers
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
400040	ELISA Tracer Dye	1 vial	1 vial
400042	ELISA 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) 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 C<sub>4</sub> 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. Adjustable pipettes and a repeating pipettor.
3. A source of 'UltraPure' water. Water used to prepare all ELISA 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 ELISA. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*

## INTRODUCTION

### Background

The leukotrienes (LTs) were discovered in 1979 as a group of acute inflammatory mediators derived from arachidonic acid in leukocytes.<sup>1,2</sup> Their biosynthesis was shown to proceed via the 5-lipoxygenase (5-LO) pathway. LT biosynthesis has subsequently been demonstrated in other bone marrow-derived cells expressing 5-LO including eosinophils, mast cells, and macrophages.

5-LO converts arachidonic acid into  $LTA_4$  with 5(S)-HpETE as an intermediate. The conjugation of glutathione to  $LTA_4$  results in the formation of  $LTC_4$ .  $LTC_4$  is rapidly metabolized to  $LTD_4$  and  $LTE_4$  as shown in Figure 1 (see page 7).<sup>3</sup> This metabolism is essentially complete within 10 minutes in the human lung.  $LTC_4$ ,  $LTD_4$ , and  $LTE_4$  are collectively referred to as cysteinyl LTs (CysLTs).

### About This Assay

Cayman's  $LTC_4$  ELISA Kit is a competitive assay that can be used for quantification of  $LTC_4$  in culture media and other sample matrices. The assay has a range from 17.1-5,000 pg/ml and a sensitivity (80% B/B<sub>0</sub>) value of approximately 40 pg/ml.

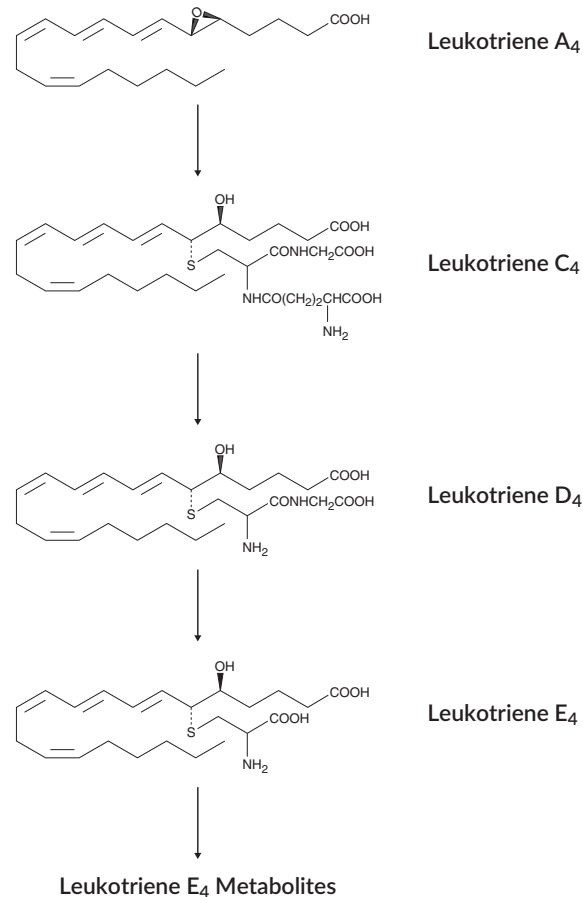


Figure 1. The formation of CysLTs

## Description of AChE Competitive ELISAs<sup>4,5</sup>

This assay is based on the competition between LTC<sub>4</sub> and an LTC<sub>4</sub>-acetylcholinesterase (AChE) conjugate (LTC<sub>4</sub> tracer) for a limited amount of LTC<sub>4</sub> antiserum. Because the concentration of the LTC<sub>4</sub> tracer is held constant while the concentration of LTC<sub>4</sub> varies, the amount of LTC<sub>4</sub> tracer that is able to bind to the LTC<sub>4</sub> antiserum will be inversely proportional to the concentration of LTC<sub>4</sub> in the well. This antibody-LTC<sub>4</sub> 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 LTC<sub>4</sub> tracer bound to the well, which is inversely proportional to the amount of free LTC<sub>4</sub> present in the well during the incubation; or

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

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

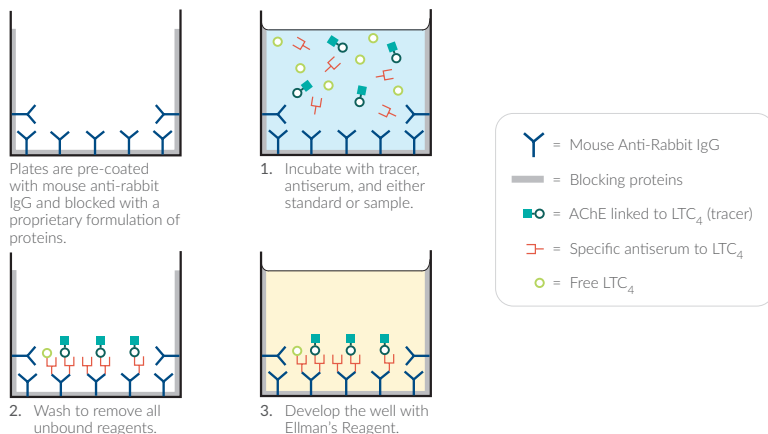


Figure 2. Schematic of the AChE ELISA

## 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, including NSB 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. Do not forget to subtract the Blank absorbance values.

**B<sub>0</sub> (Maximum Binding):** maximum amount of the tracer that the antibody can bind in the absence of free analyte.

**%B/B<sub>0</sub> (%Bound/Maximum Bound):** ratio of the absorbance of a particular sample or standard well to that of the maximum binding (B<sub>0</sub>) well.

**Standard Curve:** a plot of the %B/B<sub>0</sub> 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.

**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<sub>0</sub>) value of the tested molecule to the mid-point (50% B/B<sub>0</sub>) value of the primary analyte when each is measured in assay buffer using the following formula:

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

## PRE-ASSAY PREPARATION

*NOTE: Water used to prepare all ELISA 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 ELISA. 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. ELISA Buffer 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. *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

This assay has been validated with tissue culture supernatants. Other sample types should be checked for interference before embarking on a large number of measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample that fall within the linear range of the standard curve (*i.e.*, between ~20-80% B/B<sub>0</sub>). If the two dilutions show good correlation (differ by 20% or less) in the final calculated LTC<sub>4</sub> concentration, the sample is appropriate for this assay.

Proper sample storage and preparation are essential for consistent and accurate results. Please read this section thoroughly before beginning the assay.

### General Precautions

- All samples must be free of organic solvents prior to assay.
- AEB SF (Pefabloc SC<sup>®</sup>) and PMSF inhibit acetylcholinesterase. Samples containing these protease inhibitors should not be used in this 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 IgG plate. We do not recommend the use of rabbit samples in this assay.

### Tissue Culture Supernatants

Cultured cells synthesizing LTC<sub>4</sub> will generally release it into the medium where it will accumulate without further metabolism. Consequently, analysis of these fluids is the optimal application of this assay. For best results, the same culture medium should be used to prepare the standard curve and for dilution of samples.

Arachidonic acid displays a small, but measurable, cross reactivity with the antiserum used in this kit (cross reactivity of 0.00029%). If exogenous arachidonic acid is added to cultured cells, the samples should be diluted appropriately to eliminate interference from arachidonic acid. For example, culture medium containing 10 µg/ml of arachidonic acid should be diluted at least 1:100 prior to assay.

## Preparation of Assay-Specific Reagents

### Leukotriene C<sub>4</sub> ELISA Standard

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol several times. Using the equilibrated pipette tip, transfer 200 µl of the LTC<sub>4</sub> ELISA Standard (Item No. 420214) into a clean test tube, then dilute with 800 µl UltraPure water. The concentration of this solution (the bulk standard) will be 50 ng/ml.

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

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl ELISA Buffer to tube #1 and 500 µl ELISA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (50 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 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.

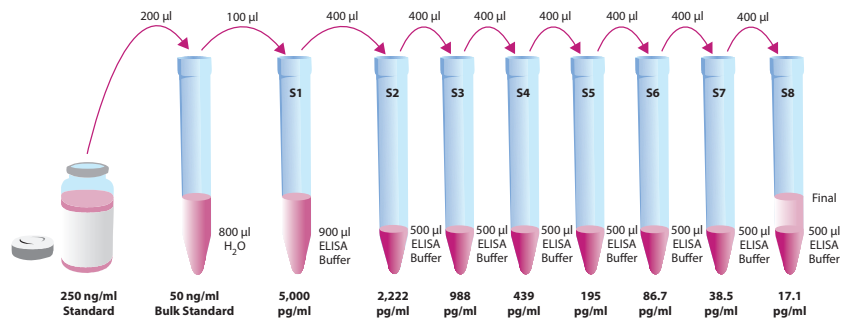


Figure 3. Preparation of the LTC<sub>4</sub> standards

### Leukotriene C<sub>4</sub> AChE Tracer

Reconstitute the LTC<sub>4</sub> AChE Tracer as follows:

**100 dtn LTC<sub>4</sub> AChE Tracer (96-well kit; Item No. 401070):** Reconstitute with 6 ml ELISA Buffer.

OR

**500 dtn LTC<sub>4</sub> AChE Tracer (480-well kit; Item No. 401070):** Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted LTC<sub>4</sub> 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).

### Leukotriene C<sub>4</sub> ELISA Antiserum

Reconstitute the LTC<sub>4</sub> ELISA Antiserum as follows:

**100 dtn LTC<sub>4</sub> ELISA Antiserum (96-well kit; Item No. 401072):** Reconstitute with 6 ml ELISA Buffer.

OR

**500 dtn LTC<sub>4</sub> ELISA Antiserum (480-well kit; Item No. 401072):** Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted LTC<sub>4</sub> ELISA Antiserum at 4°C. It will be stable for at least two 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 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).

## 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 (B<sub>0</sub>), 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 18, for more details). We suggest you record the contents of each well on the template sheet provided (see page 26).

	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 <sub>0</sub>	S5	S5	5	5	5	13	13	13	21	21	21
F	B <sub>0</sub>	S6	S6	6	6	6	14	14	14	22	22	22
G	B <sub>0</sub>	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<sub>0</sub> - Maximum Binding  
 S1-S8 - Standards 1-8  
 1-24 - Samples

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

Add 100 µl ELISA Buffer to NSB wells. Add 50 µl ELISA Buffer to B<sub>0</sub> wells. If culture medium was used to dilute the standard curve, substitute 50 µl of culture medium for ELISA Buffer in the NSB and B<sub>0</sub> wells (*i.e.*, add 50 µl culture medium to NSB and B<sub>0</sub> wells and 50 µl ELISA Buffer to NSB wells).

#### 2. Leukotriene C<sub>4</sub> 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 C<sub>4</sub> AChE Tracer

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

#### 5. Leukotriene C<sub>4</sub> ELISA Antiserum

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



Well	ELISA Buffer	Standard/ Sample	Tracer	Antiserum
Blk	-	-	-	-
TA	-	-	5 $\mu$ l (at devel. step)	-
NSB	100 $\mu$ l (or 50 $\mu$ l ELISA Buffer + 50 $\mu$ l media)	-	50 $\mu$ l	-
B <sub>0</sub>	50 $\mu$ l (or 50 $\mu$ l media)	-	50 $\mu$ l	50 $\mu$ l
Std/Sample	-	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l

**Table 1. Pipetting summary**

### Incubation of the Plate

Cover each plate with plastic film (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 run on different days.*

2. Empty the wells and rinse five times with Wash Buffer.
3. Add 200  $\mu$ l of Ellman's Reagent to each well.
4. Dilute the Leukotriene C<sub>4</sub> AChE Tracer 1:10 with ELISA Buffer (for example, 50  $\mu$ l Tracer into 450  $\mu$ l ELISA Buffer). Add 5  $\mu$ l of diluted 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.*, B<sub>0</sub> wells  $\geq 0.3$  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. 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 B<sub>0</sub> wells have reached a minimum of 0.3 A.U. (blank subtracted). The plate should be read when the absorbance of the B<sub>0</sub> wells are in the range of 0.3-1.5 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.

## 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<sub>0</sub> versus log concentration using a four-parameter logistic fit or as logit B/B<sub>0</sub> 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](http://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<sub>0</sub> wells.
3. Subtract the NSB average from the B<sub>0</sub> average. This is the corrected B<sub>0</sub> or corrected maximum binding.
4. Calculate the B/B<sub>0</sub> (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<sub>0</sub> (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B<sub>0</sub> 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; the corrected B<sub>0</sub> 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 20). 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 24 for **Troubleshooting**).*

#### Plot the Standard Curve

Plot %B/B<sub>0</sub> for standards S1-S8 versus LTC<sub>4</sub> 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/B<sub>0</sub> in this calculation.*

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

Plot the data as logit (B/B<sub>0</sub>) versus log concentrations and perform a linear regression fit.

#### Determine the Sample Concentration

Calculate the B/B<sub>0</sub> (or %B/B<sub>0</sub>) 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/B<sub>0</sub> 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.*

## Performance Characteristics

### Sample Data

The standard curve presented here was generated by running the assay multiple times across several days and represents 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.

	Raw Data	Corrected
Total Activity	2.596	2.496
NSB	0.170	0.070
B <sub>0</sub>	0.676	0.576

Dose (pg/ml)	Raw Data	Corrected	%B/B <sub>0</sub>
5,000	0.141	0.041	6.9
2,222	0.175	0.075	9.6
988	0.230	0.130	22.5
439	0.306	0.206	35.0
195	0.385	0.285	49.4
86.7	0.483	0.383	66.8
38.5	0.571	0.471	82.2
17.1	0.592	0.492	86.1

Table 2. Typical results

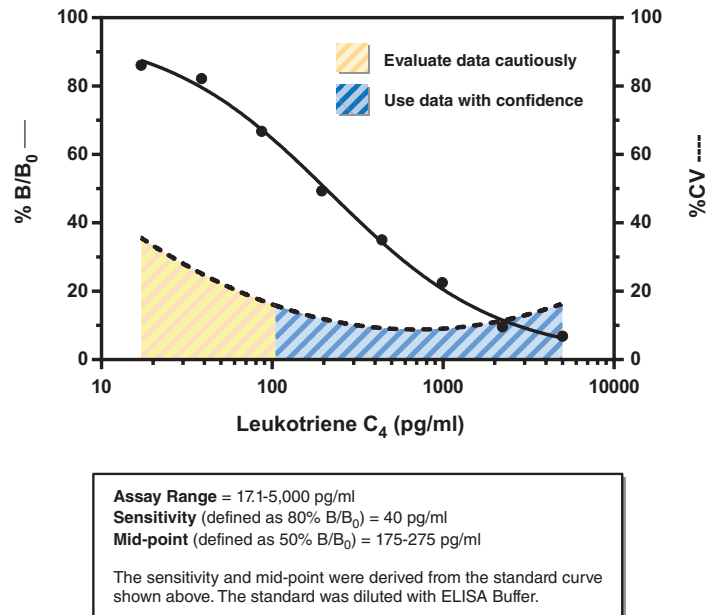


Figure 5. Typical standard curve

### Precision:

Tissue culture samples containing a high, medium, or low level of LTC<sub>4</sub> were measured 60 times each using a single set of reagents. The calculated %CV is reported as intra-assay variance. A separate series of samples containing a high, medium, or low level of LTC<sub>4</sub> were measured four times each using eight independent sets of reagents. The calculated %CV is reported as inter-assay variance.

Level	Average (pg/ml)	Intra-assay variation (%CV)	Average (pg/ml)	Inter-assay variation (%CV)
High	269.11	8.5	306.07	19.5
Medium	174.96	11.6	186.20	19.0
Low	68.14	11.4	53.02	21.5

Table 3. Tissue culture sample validation

### Cross Reactivity:

Compound	Cross Reactivity
Leukotriene C <sub>4</sub>	100%
Leukotriene C <sub>4</sub> methyl ester	37%
Leukotriene B <sub>4</sub>	1.2%
Leukotriene E <sub>4</sub>	0.16%
14,15-Leukotriene C <sub>4</sub>	0.03%
Arachidonic Acid	0.00029%
L-Glutathione	<0.01%
Leukotriene D <sub>4</sub>	<0.01%
N-acetyl Leukotriene E <sub>4</sub>	<0.01%
tetranor-PGEM	<0.01%
tetranor-PGFM	<0.01%

Table 4. Cross Reactivity of LTC<sub>4</sub> ELISA

## RESOURCES

### Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	Replace activated carbon filter or change source of UltraPure Water
High NSB (>10% of B <sub>0</sub> )	A. Poor washing B. Exposure of NSB wells to specific antibody	Rewash plate and redevelop
Very low B <sub>0</sub>	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.e., more than 20% difference)	Interfering substances are present	Samples may not be suitable for use in this assay
Only Total Activity (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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