Leukotriene C_4 ELISA Kit

Item No. 501070

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
401072	Leukotriene C_4 ELISA Antiserum	1 vial/100 dtn	1 vial/500 dtn
401070	Leukotriene C_4 AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
420214	Leukotriene C_4 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_4 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

Leukotriene C_4 (LTC_4) is a potent inflammatory mediator formed *via* the 5-lipoxygenase (5-LO) pathway in leukocytes and other 5-LO-expressing cells, including mast cells, eosinophils, and macrophages.[1-3] Upon cellular stimulation or injury, 5-LO converts arachidonic acid into LTA_4 with 5(S)-HpETE as an intermediate.[1] The subsequent conjugation of glutathione to LTA_4 by LTC_4 synthase results in LTC_4 formation. LTC_4 is rapidly metabolized by γ-glutamyl transpeptidases to LTD_4, which is then converted to LTE_4 by dipeptidases.[1,3] LTC_4 is also produced *via* transcellular metabolism in platelets, which lack 5-LO but express LTC_4 synthase, and in endothelial and smooth muscle cells by microsomal glutathione S-transferase 2 (MGST2).[4,5] LTC_4 acts on cysteinyl leukotriene 1 ($CysLT_1$) and $CysLT_2$ receptors to induce bronchoconstriction and vasoconstriction and is recognized as a paracrine mediator pertinent to asthma and allergic diseases.[6-8]

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, an average sensitivity (80% B/B_0) of 21.2 pg/ml, and a lower limit of detection (LLOD) of 7.4 pg/ml.

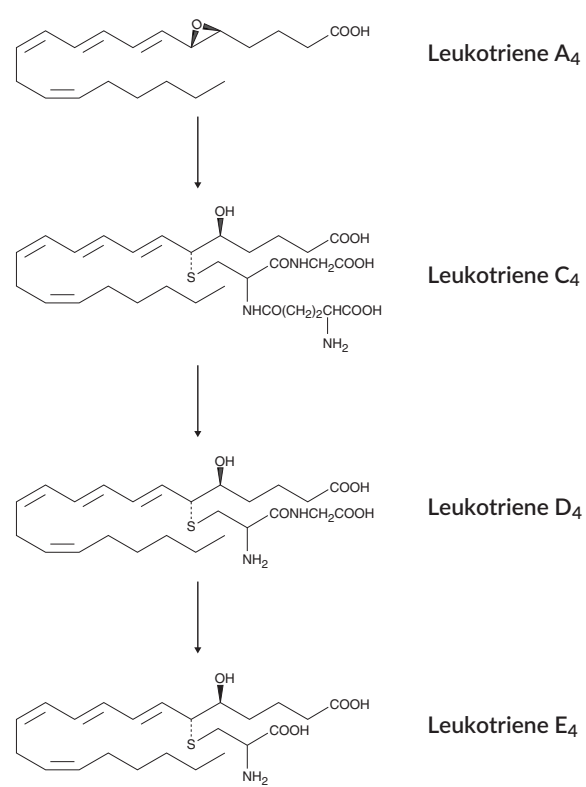


Figure 1. The formation of CysLTs

Principle of the Assay

This assay is based on the competition between LTC_4 and an LTC_4-acetylcholinesterase (AChE) conjugate (LTC_4 tracer) for a limited amount of LTC_4 antiserum. Because the concentration of the LTC_4 tracer is held constant while the concentration of LTC_4 varies, the amount of LTC_4 tracer that is able to bind to the LTC_4 antiserum will be inversely proportional to the concentration of LTC_4 in the well. This antibody-LTC_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 LTC_4 tracer bound to the well, which is inversely proportional to the amount of free LTC_4 present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound } LTC_4 \text{ Tracer}] \propto 1/[LTC_4]$$

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

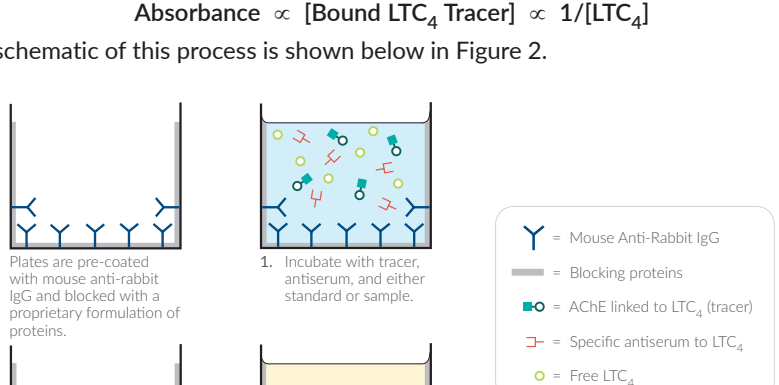


Figure 2. Schematic of the AChE ELISA

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 LTC_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:

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

LLOD (Lower Limit of Detection): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

PRE-ASSAY PREPARATION

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 (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. *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 (1X) Preparation**

   5 ml vial Wash Buffer Concentrate (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 Concentrate (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 (1X) 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 evaluated 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_0). If the two dilutions show good correlation (differ by 20% or less) in the final calculated LTC_4 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.
- AEBSF (Pefabloc SC®) 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_4 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.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Leukotriene C_4 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_4 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 (1X), culture medium should be used in place of ELISA Buffer (1X) for dilution of the standard curve.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #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 (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.

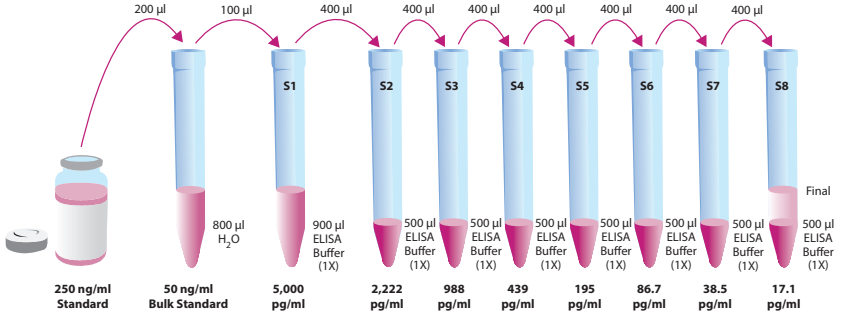


Figure 3. Preparation of the LTC_4 standards

Leukotriene C_4 AChE Tracer

Reconstitute the LTC_4 AChE Tracer as follows:

100 dtn LTC_4 AChE Tracer (96-well kit; Item No. 401070): Reconstitute with 6 ml ELISA Buffer (1X).

OR

500 dtn LTC_4 AChE Tracer (480-well kit; Item No. 401070): Reconstitute with 30 ml ELISA Buffer (1X).

Store the reconstituted LTC_4 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). Do not store tracer with dye.

Leukotriene C_4 ELISA Antiserum

Reconstitute the LTC_4 ELISA Antiserum as follows:

100 dtn LTC_4 ELISA Antiserum (96-well kit; Item No. 401072): Reconstitute with 6 ml ELISA Buffer (1X).

OR

500 dtn LTC_4 ELISA Antiserum (480-well kit; Item No. 401072): Reconstitute with 30 ml ELISA Buffer (1X).

Store the reconstituted LTC_4 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). Do not store tracer 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 blanks (Blk), two non-specific binding wells (NSB), two maximum binding wells (B_0), 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 21, for more details). We suggest you record the contents of each well on the template sheet provided (see page 29).

	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 4. Sample plate format

Performing the Assay

Pipetting Hints

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

Addition of the Reagents

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

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

2. **Leukotriene C_4 ELISA Standard**

   Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. **Samples**

   Add 50 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. **Leukotriene C_4 AChE Tracer**

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

5. **Leukotriene C_4 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 µl (at devl. step)	-
NSB	100 µl (or 50 µl ELISA Buffer (1X) + 50 µl media)	-	50 µl	-
B_0	50 µl (or 50 µl media)	-	50 µl	50 µl
Std/Sample	-	50 µl	50 µl	50 µl

Table 1. Pipetting summary

Incubation of the Plate

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

Development of the Plate

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

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

   OR

   250 dtn vial Ellman's Reagent (480-well kit; Item No. 400050): Reconstitute with 50 ml of ultrapure water.

NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day it is prepared; protect the Ellman's Reagent from light when not in use. Extra vials of the reagent have been provided should a plate need to be re-developed or multiple assays run on different days.

2. Empty the wells and rinse five times with Wash Buffer (1X).
3. Add 200 µl of Ellman's Reagent to each well.
4. Dilute the Leukotriene C_4 AChE Tracer 1:10 with ELISA Buffer (1X) (for example, 50 µl Tracer into 450 µl ELISA Buffer (1X)). Add 5 µl of diluted tracer to the Total Activity wells.
5. Cover the plate with the 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. (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_0 wells have reached a minimum of 0.3 A.U. (blank subtracted). The plate should be read when the absorbance of the B_0 wells 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_0 *versus* log concentration using a four-parameter logistic fit or as logit B/B_0 *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B_0 for a logistic four-parameter fit, multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system. Only the linear part of this standard curve should be used in calculations.

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* LTC_4 concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be lineraized 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 concentration or dilution of the sample prior to the addition to the well.* Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve.

Performance Characteristics

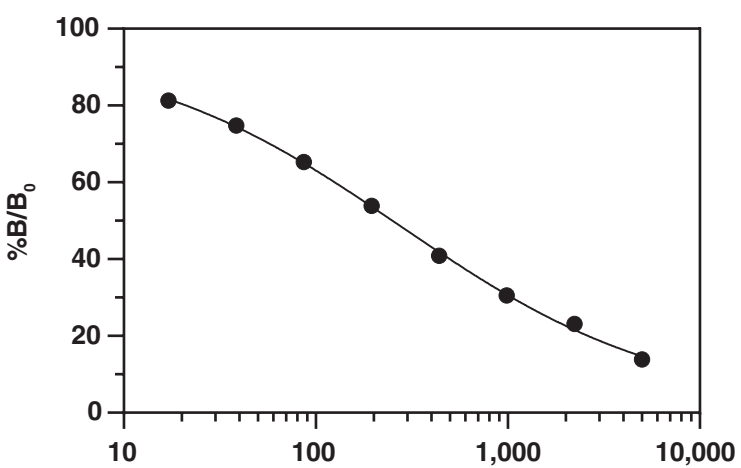
Sample Data

The standard curve presented here 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.

Absorbance at 414 nm (90 minutes)

LTC_4 (pg/ml) and Controls	Blank-subtracted Absorbance	NSB-corrected Absorbance	%B/B_0	%CV* Inter-Assay Precision	%CV* Intra-Assay Precision
TA	0.209	--	--	--	--
NSB	0.000	--	--	--	--
B_0	0.601	0.601	--	--	--
5,000	0.083	0.083	13.9	11.8	6.0
2,222	0.138	0.138	23.1	7.6	5.2
988	0.183	0.183	30.6	6.0	5.9
439	0.245	0.245	40.9	8.1	3.8
195	0.323	0.323	53.9	7.8	5.2
86.7	0.392	0.392	65.2	12.8	7.1
38.5	0.449	0.449	74.8	14.4	10.6
17.1	0.489	0.489	81.3	16.7	5.8

Table 2. Typical results
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.



Assay Range = 17.1-5,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 21.2 pg/ml
Mid-point (defined as 50% B/B_0) = 251 pg/ml
Lower Limit of Detection (LLOD) = 7.4 pg/ml

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

Figure 5. Typical standard curve

Precision:

Tissue culture samples containing a high, medium, or low level of LTC_4 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_4 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_4	100%
Leukotriene C_4 methyl ester	37%
Leukotriene B_4	1.2%
Leukotriene E_4	0.16%
14,15-Leukotriene C_4	0.03%
Arachidonic Acid	0.00029%
L-Glutathione	<0.01%
Leukotriene D_4	<0.01%
N-acetyl Leukotriene E_4	<0.01%
tetranor-PGEM	<0.01%
tetranor-PGFM	<0.01%

Table 4. Cross Reactivity of LTC_4 ELISA

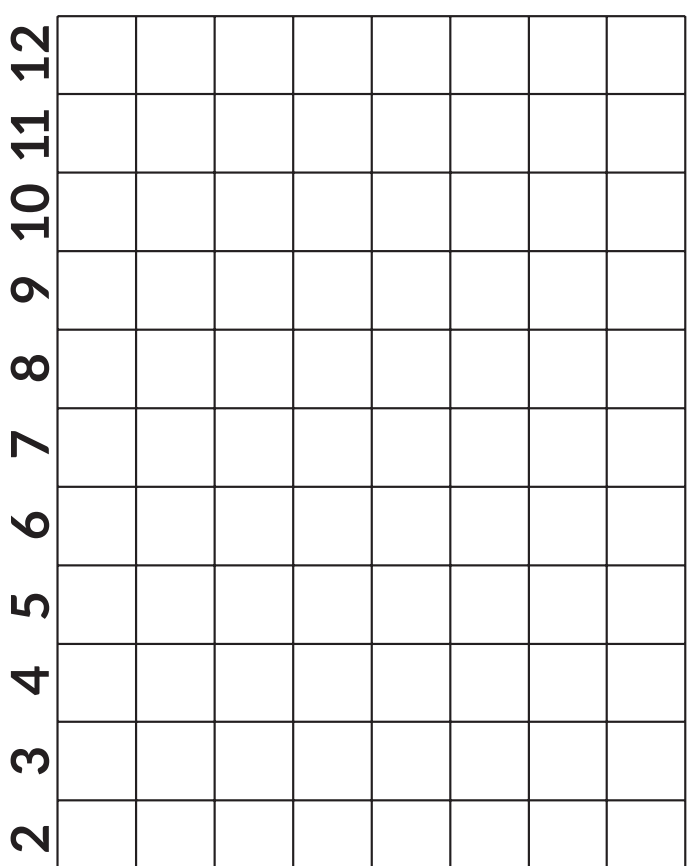
RESOURCES

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_0)	A. Poor washing B. Exposure of NSB wells to specific antibody	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.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

References

1. Miyata, J., Fukunaga, K., Kawashima, Y., *et al*. Cysteinyl leukotriene metabolism of human eosinophils in allergic disease. *Allergol. Int*. **69(1)**, 28-34 (2020).
2. Saier, L. and Peyruchaud, O. Emerging role of cysteinyl LTs in cancer. *Br. J. Pharmacol*.. **179(22)**, 5036-5055 (2022).
3. Tsai, M.-J., Chang, W.-A., Chuang, C.-H., *et al*. Cysteinyl leukotriene pathway and cancer. *Int. J. Mol. Sci.* **23(1)**, 120 (2022).
4. Penrose, J.F. LTC_4 synthase. Enzymology, biochemistry, and molecular characterization. *Clin. Rev. Allergy Immunol.* **17**, 133-152 (1999).
5. McNamara, M.J.C., Schmitt, J.D., Wykle, R.L., *et al.* 1-O-Hexadecyl-2-acetyl-sn-glycerol stimulates differentiation of HL-60 human promyelocytic leukemia cells to macrophage-like cells. *Biochem. Biophys. Res. Commun.* **122**, 824-830 (1984).
6. Murphy, R.C. and Gijón, M. Biosynthesis and metabolism of leukotrienes. *Biochem. J.* **405**, 379-395 (2007).
7. Bernström, K. and Hammarström, S. Metabolism of leukotriene D by porcine kidney. *J. Biol. Chem.* **256(18)**, 9579-9582 (1981).
8 Bandeira-Melo, C., Bozza, P.T., and Weller, P.F. The cellular biology of eosinophil eicosanoid formation and function. *J. Allergy Clin. Immunol.* **109(3)**, 393-400 (2002).



NOTES

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