Cysteinyl Leukotriene Express ELISA Kit

Item No. 10009291

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
10011322	Cysteinyl Leukotriene Express ELISA Monoclonal Antibody	1 vial/100 dtn	1 vial/500 dtn
400787	Cysteinyl Leukotriene Express AChE Tracer	1 vial/100 dtn	2 vials/500 dtn
420504	Cysteinyl Leukotriene Express ELISA Standard	1 vial/500 µl	1 vial/500 µl
10011325	Immunoassay Buffer A 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
400008/400009	Goat Anti-Mouse 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

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 Cysteinyl Leukotriene Express 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. An orbital microplate shaker
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 14)

INTRODUCTION

Background

Leukotriene C_4 (LTC_4), LTD_4, and LTE_4, collectively referred to as cysteinyl leukotrienes (CysLTs), are potent inflammatory mediators 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] CysLTs bind to three CysLT receptor subtypes with varying potencies: LTC_4 binds primarily to $CysLT_1$ and $CysLT_3$ receptors, while LTD_4 and LTE_4 both bind to the $CysLT_1$ and $CysLT_2$ receptors, although LTE_4 binds only weakly.[3] Through these receptors, CysLTs induce bronchoconstriction, increase vascular permeability, and constrict coronary arteries, among other activities.[4,5] They are pathogenic mediators of allergic inflammation, asthma, and cardiovascular disease.[5] CysLTs and their receptors also have roles in cancer cell proliferation, survival, migration, and invasion.[2]

About This Assay

Cayman's CysLT Express ELISA Kit is a competitive assay that can be used for quantification of CysLTs in urine, plasma, serum, lavage fluids and aspirates, culture media, and other sample matrices. The assay has a range of 7.8-1,000 pg/ml, an average sensitivity (80% B/B_0) of 24 pg/ml, and a lower limit of detection (LLOD) of 6.4 pg/ml.

NOTE: This kit measures all CysLTs. For measurement of specific CysLTs, please see Cayman Chemical's Leukotriene E_4 ELISA Kit (Item No. 501060) and Leukotriene C_4 ELISA Kit (Item No. 501070).

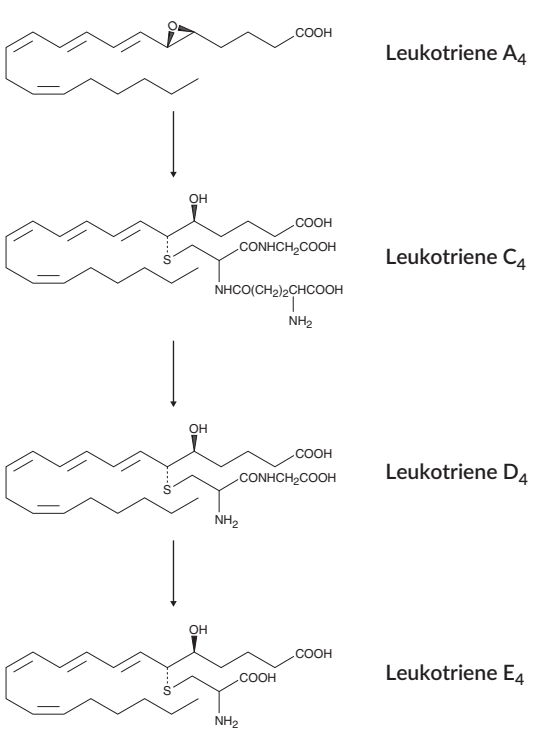


Figure 1. The formation of CysLTs

Principle of This Assay

This assay is based on the competition between free CysLTs and a CysLT-acetylcholinesterase (AChE) conjugate (CysLT Express AChE Tracer) for a limited number of CysLT monoclonal antibody binding sites. Because the concentration of the CysLT Express AChE Tracer is held constant while the concentration of free CysLTs varies, the amount of CysLT Express AChE Tracer that is able to bind to the CysLT Express ELISA Monoclonal Antibody will be inversely proportional to the concentration of free CysLTs in the well. This antibody-CysLT complex binds to a goat anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly with a λ_{max} value of 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of CysLT Express AChE Tracer bound to the well, which is inversely proportional to the amount of free CysLTs present in the well during the incubation, as described in the equation:

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

A schematic of this process is shown in Figure 2, on page 10.

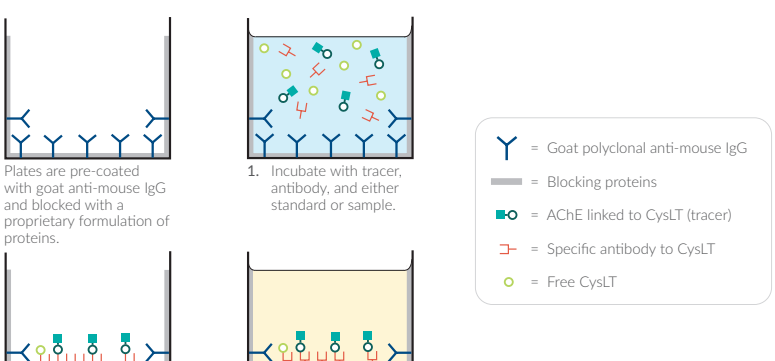


Figure 2. Schematic of the ELISA

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 the non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the 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) 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): 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_0) value of the tested molecule to the mid-point (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

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for at least two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. **Immunoassay Buffer A (1X) Preparation**

   Dilute the contents of one vial of Immunoassay Buffer A Concentrate (10X) (Item No. 10011325) 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

Heterogeneous mixtures such as lavage fluids and aspirates may contain contaminants, which can interfere in the assay. It is best to check for interference before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to at least two different dilutions within the linear portion of the standard curve. If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated CysLT concentration, purification is not required.

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 containing BSA or FBS may interfere with this assay. AEBSF (Pefabloc SC®) and PMSF inhibit acetylcholinesterase. Samples containing these protease inhibitors should not be used in this assay.
- Samples of mouse and rat origin may contain antibodies that interfere with the assay by binding the goat anti-mouse IgG-coated plate. We recommend that all mouse and rat samples be purified prior to use in the 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 leukotriene metabolism is incomplete in these circumstances, substantial amounts of LTC_4, LTD_4, and LTE_4 may be present (*e.g.*, bronchoalveolar lavage fluid from asthmatic subjects may contain 700-1,000 pg/ml CysLTs composed mainly of LTC_4 and LTD_4).[6] Consequently, analysis of these fluids is the optimal application of this assay. Due to the complex nature of these samples there may be significant interference in unpurified samples.[6,7]

Lavage fluids and aspirates should be diluted at least 1:4 with Immunoassay Buffer A (1X) prior to the assay to eliminate interference.

Urine

CysLTs are excreted in urine as intact LTE_4 and LTE_4 metabolites. The levels of LTE_4 reported in urine vary depending on the method of measurement. Values obtained from LC-MS (25-40 pg/mg creatinine) or HPLC followed by ELISA (20-160 pg/mg creatinine) are generally lower than values from ELISA measurements alone (200-1,500 pg/mg creatinine).[8-10] It is recommended that the values obtained in urine samples be standardized to the creatinine levels. To measure creatinine, Cayman offers the Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701) or the Creatinine ELISA Kit (Item 502330).

Urine should be diluted at least 1:2 with Immunoassay Buffer A (1X) prior to the assay. Some samples may require higher dilution to be in range of the standard curve.

Plasma

Collect blood in vacutainers containing heparin, citrate, or EDTA for plasma samples. To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes. Transfer the top plasma layer into a clean test tube, without disturbing the white buffy layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. Purification of plasma samples before testing is strongly advised (see **Protein Precipitation Protocol** on page 17).

Serum

Collect blood in vacutainers without a coagulant for serum samples. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1,000-2,000 x g for 15-30 minutes. Transfer the serum layer into a clean test tube. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C. Purification of serum samples before testing is strongly advised (see **Protein Precipitation Protocol** on page 17).

Culture Medium

Cultured cells synthesizing LTC_4 will generally release it into the medium where it will accumulate without further metabolism. Culture medium, in general, can be assayed following dilution with Immunoassay Buffer A (1X) greater than 10-fold. If the samples are not diluted greater than 10-fold in Immunoassay Buffer A (1X), the standard curve and the samples should be diluted with culture medium.

Protein Precipitation Protocol

1. Aliquot samples into clean test tubes. At least 400 µl is recommended if assaying in several dilutions. If samples need to be concentrated, use larger volumes.
2. To precipitate proteins, add cold ethanol or acetone (approximately four times the sample volume) to each sample. Vortex to mix thoroughly.
3. Incubate samples at 4°C for five minutes, then centrifuge at 3,000-5,000 x g for 10 minutes to remove precipitated proteins.
4. Transfer the supernatants to clean test tubes and evaporate the solvent under nitrogen.
5. Resuspend the samples in Immunoassay Buffer A (1X) to their original volume, and use for ELISA analysis. Samples can be concentrated in this step by using smaller volumes of buffer compared to the original sample volumes.

Sample Matrix Properties

Parallelism

To assess parallelism, human urine, plasma, and serum were processed as described in the **Sample Preparation** section (see page 14), serially diluted with Immunoassay Buffer A (1X), and evaluated using the CysLT Express ELISA Kit. Measured concentrations were plotted as a function of the sample dilution. The results are shown below.

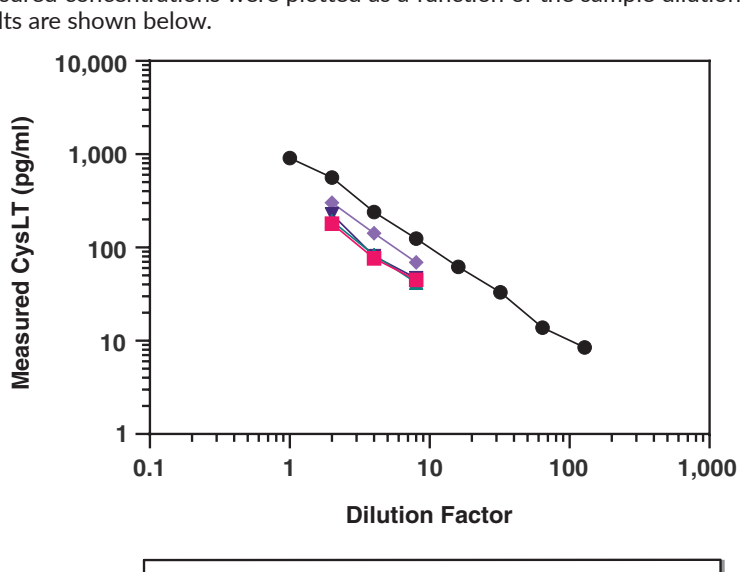


Figure 3. Parallelism of various matrices

Spike and Recovery

Human urine and plasma, were spiked with LTC_4, processed as described in the **Sample Preparation** section (see page 14), serially diluted with Immunoassay Buffer A (1X), and evaluated using the CysLT Express ELISA Kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

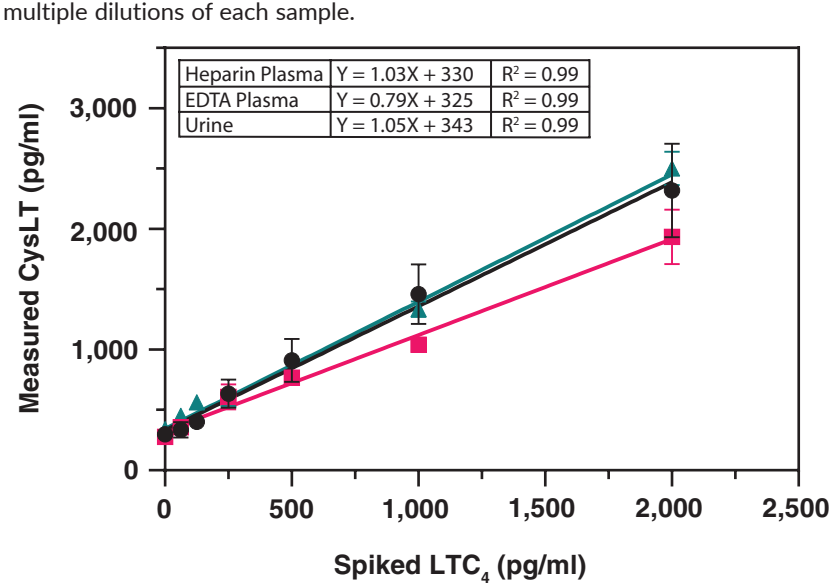


Figure 4. Spike and recovery of CysLT in various matrices

Linearity

Human urine, plasma, and serum samples were spiked with LTC_4, processed as described in the **Sample Preparation** section, serially diluted with Immunoassay Buffer A (1X), and evaluated for linearity using the CysLT Express ELISA Kit. The results are shown in Table 1, below.

Dilution Factor	Measured Concentration (pg/ml)	Linearity (%)
Spike: 2,000 pg/ml into urine		
20	2,565	100
40	2,344	91.4
80	2,596	101
Spike: 1,000 pg/ml into EDTA plasma		
5	1,081	100
10	996	92.1
20	1,064	98.4
Spike: 250 pg/ml into serum		
4	1,067	100
8	1,059	99.3
16	1,121	105

Table 1. Linearity in various matrices

Preparation of Assay-Specific Reagents

Cysteinyl Leukotriene Express ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Cysteinyl Leukotriene Express ELISA Standard (Item No. 420504) 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 that have not been diluted at least 10-fold with Immunossay Buffer A (1X), culture medium should be used in place of Immunoassay Buffer A (1X) for dilution of the standard curve.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1 through #8. Aliquot 900 µl Immunoassay Buffer A (1X) to tube #1 and 500 µl Immunoassay Buffer A (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 not be stored for more than 24 hours.

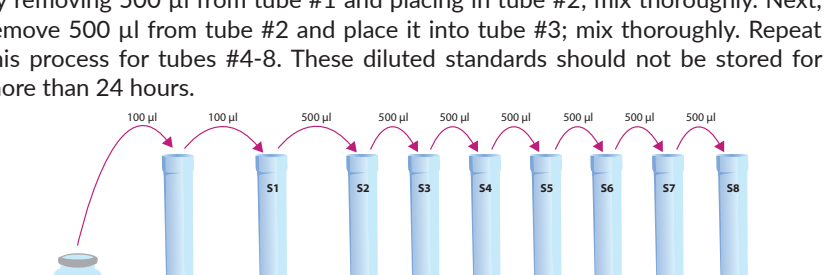


Figure 5. Preparation of the CysLT standards

Cysteinyl Leukotriene Express AChE Tracer

Reconstitute the CysLT Express AChE Tracer as follows:

100 dtn CysLT Express AChE Tracer (96-well kit; Item No. 400787): Reconstitute with 6 ml Immunoassay Buffer A (1X).

OR

500 dtn CysLT Express AChE Tracer (480-well kit; Item No. 400787): Reconstitute with 30 ml Immunoassay Buffer A (1X).

Transfer the reconstituted tracer to a polypropylene tube or vial, store at 4°C (*do not freeze*), and use within one week. A 20% surplus per vial of tracer has been included to account for any incidental losses.

NOTE: The 480-well size kit contains two 500 dtn vials of tracer so that the kit may be used for more than one week. Each vial of tracer will be stable for one week from reconstitution.

Cysteinyl Leukotriene Express ELISA Monoclonal Antibody

Reconstitute the CysLT Express ELISA Monoclonal Antibody as follows:

100 dtn CysLT Express ELISA Monoclonal Antibody (96-well kit; Item No. 10011322): Reconstitute with 6 ml Immunoassay Buffer A (1X).

OR

500 dtn CysLT Express ELISA Monoclonal Antibody (480-well kit; Item No. 10011322): Reconstitute with 30 ml Immunoassay Buffer A (1X).

Store the reconstituted CysLT Express ELISA Monoclonal Antibody at 4°C. Use within two weeks. A 20% surplus of antibody has been included to account for any incidental losses.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two Blk, two NSB, and two B_0 wells, and an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 6, 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 28, for more details). We suggest you record the contents of each well on the template sheet provided (see page 37).

	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 6. 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. **Immunoassay Buffer A (1X)**

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

2. **Cysteinyl Leukotriene Express ELISA Standard**

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

3. **Samples**

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

4. **Cysteinyl Leukotriene Express AChE Tracer**

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

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

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

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

Table 2. Pipetting summary

Incubation of the Plate

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

Development of the Plate

1. Reconstitute Ellman's Reagent 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 be run on different days.

2. Empty the wells and rinse five times with 300 µl of Wash Buffer (1X).
3. Add 200 µl of Ellman's Reagent to each well.
4. Add 5 µl of the reconstituted tracer to the TA wells.
5. Cover the plate with the 96-Well Cover Sheet. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (*i.e.*, B_0 wells ≥0.3 A.U. (Blk subtracted)) in 60-90 minutes.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings. 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 (1X) 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. (Blk subtracted). The plate should be read when the absorbance of the B_0 wells is in the range of 0.3-2.0 A.U. (Blk subtracted). If the absorbance of the wells exceeds 2.0 A.U., wash the plate, add fresh Ellman's Reagent, and let it develop again.

ANALYSIS

Many plate readers come with data reduction software that 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/analysis/elisa) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

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

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

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

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

Plot the Standard Curve

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

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B_0 in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B_0 (or %B/B_0) value for each sample. Determine the concentration of each sample using the equation obtained from the standard curve plot. *NOTE: Remember to account for any dilution or concentration of the sample prior to its addition to the well.* Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve.

Performance Characteristics

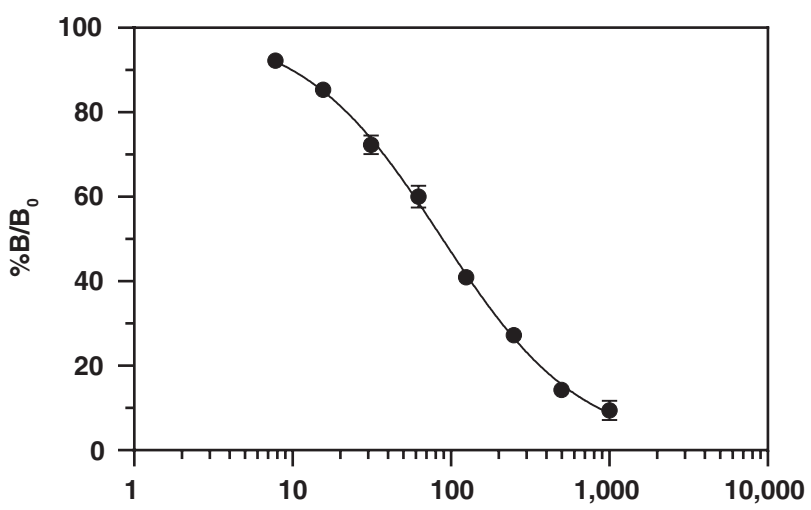
Representative Data

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 in Table 3 on page 31 to determine the values of your samples.

Absorbance at 414 nm (60 minutes)

CysLT Standards (pg/ml) and Controls	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	%B/B_0	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
TA	1.008	--	--	--	--
NSB	0.003	--	--	--	--
B_0	0.880	0.877	--	--	--
1,000	0.086	0.083	9.5	11.1	6.4
500	0.129	0.126	14.4	8.5	9.8
250	0.242	0.239	27.3	10.1	11.2
125	0.362	0.359	40.9	10.7	6.7
62.5	0.530	0.527	60.1	12.4	5.0
31.3	0.637	0.634	72.3	10.8	6.9
15.6	0.751	0.748	85.3	12.4	11.0
7.8	0.812	0.809	92.2	11.9	10.2

Table 3. Typical results
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve



Assay Range = 7.8-1,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 22 pg/ml
Mid-point (defined as 50% B/B_0) = 89 pg/ml
Lower Limit of Detection (LLOD) = 6.4 pg/ml

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

Figure 7. 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
2,013	6.2
985	3.4
242	7.5

Table 4. Intra-assay precision

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

Matrix Control (pg/ml)	%CV
2,499	2.7
1,018	11.0
267	10.4

Table 5. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity
N-methyl Leukotriene C_4	113%
Leukotriene D_4	105%
Leukotriene C_4	100%
Leukotriene E_4	77.1%
Arachidonic Acid	0.03%
5(6)-DiHETE	0.015%
5(S),15(S)-DiHETE	<0.01%
5(S)-HETE	<0.01%
5(R)-HETE	<0.01%
12(S)-HETE	<0.01%
12(R)-HETE	<0.01%
15(S)-HETE	<0.01%
15(R)-HETE	<0.01%
Leukotriene B_4	<0.01%
Prostaglandin D_2	<0.01%
Prostaglandin E_2	<0.01%

Table 6. Cross reactivity of the CysLT Express 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 antibody
Very low B_0 (<0.3 A.U. (Blk subtracted))	A. Trace organic contaminants in the water source B. Plate requires additional development time C. Dilution error in preparing reagents
Shift in standard curve	A. Dilution error in standard preparation B. Standard has degraded
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present
Only TA wells develop	A. Trace organic contaminants in the water source B. Tracer or antibody weren't added to the wells.

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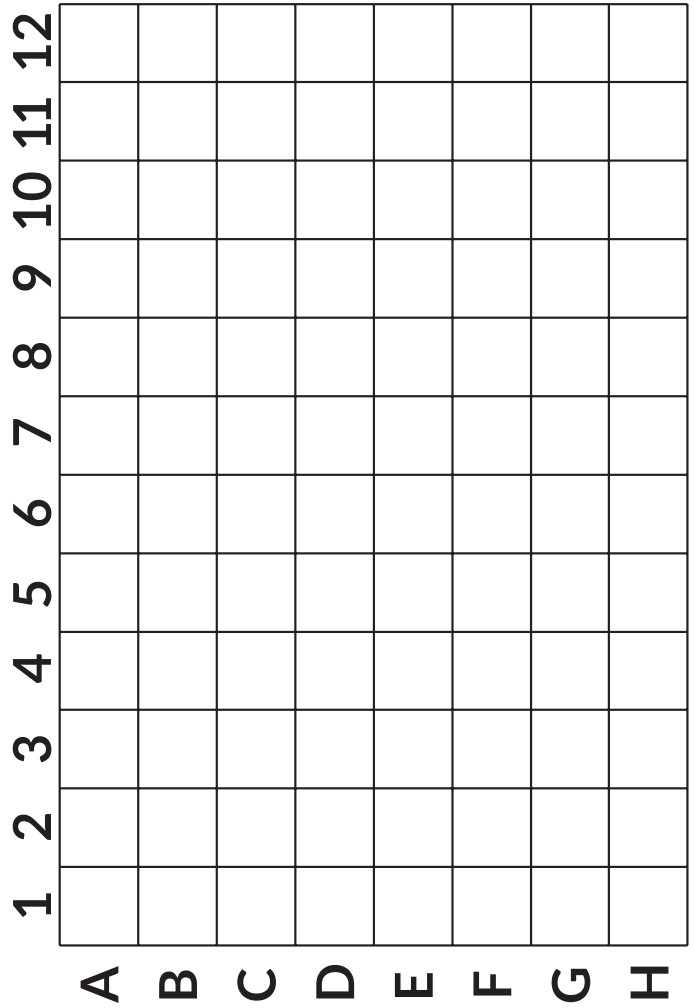

NOTES

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