Cysteinyl Leukotriene EIA Kit

Item No. 500390
TABLE OF CONTENTS

GENERAL INFORMATION  3 Materials Supplied  4 Precautions  4 If You Have Problems  4 Storage and Stability  4 Materials Needed but Not Supplied

INTRODUCTION  5 Background  5 About This Assay  7 Description of ACE™ Competitive EIAs  8 Biochemistry of Acetylcholinesterase  10 Definition of Key Terms

PRE-ASSAY PREPARATION  11 Buffer Preparation  12 Sample Preparation

ASSAY PROTOCOL  16 Preparation of Assay-Specific Reagents  18 Plate Set Up  19 Performing the Assay

ANALYSIS  22 Calculations  24 Performance Characteristics

RESOURCES  28 Troubleshooting  29 References  30 Related Products  30 Warranty and Limitation of Remedy  31 Plate Template  32 Notes

GENERAL INFORMATION

Materials Supplied

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>96 wells Quantity/Size</th>
<th>480 wells Quantity/Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>400392</td>
<td>Cysteinyl Leukotriene EIA Monoclonal Antibody</td>
<td>1 vial/100 dtn</td>
<td>1 vial/500 dtn</td>
</tr>
<tr>
<td>400390</td>
<td>Cysteinyl Leukotriene AChE Tracer</td>
<td>1 vial/100 dtn</td>
<td>1 vial/500 dtn</td>
</tr>
<tr>
<td>420504</td>
<td>Cysteinyl Leukotriene EIA Standard</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
<tr>
<td>10011325</td>
<td>Immunoassay Buffer A Concentrate (10X)</td>
<td>2 vials/10 ml</td>
<td>4 vials/10 ml</td>
</tr>
<tr>
<td>400062</td>
<td>Wash Buffer Concentrate (400X)</td>
<td>1 vial/5 ml</td>
<td>1 vial/12.5 ml</td>
</tr>
<tr>
<td>400035</td>
<td>Tween 20</td>
<td>1 vial/3 ml</td>
<td>1 vial/3 ml</td>
</tr>
<tr>
<td>400008/400009</td>
<td>Goat Anti-Mouse IgG Coated Plate</td>
<td>1 plate</td>
<td>5 plates</td>
</tr>
<tr>
<td>400012</td>
<td>96-Well Cover Sheet</td>
<td>1 cover</td>
<td>5 covers</td>
</tr>
<tr>
<td>400050</td>
<td>Ellman’s Reagent</td>
<td>3 vials/100 dtn</td>
<td>6 vials/250 dtn</td>
</tr>
<tr>
<td>400040</td>
<td>EIA Tracer Dye</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
<tr>
<td>400042</td>
<td>EIA Antiserum Dye</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

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 12).

INTRODUCTION

Background

The leukotrienes (LTs) were discovered in 1979 as a group of acute inflammatory mediators derived from arachidonic acid in leukocytes.1,2 Their biosynthesis was shown to proceed via the 5-lipoxigenase (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 LTA4 with 5(S)-HpETE as an intermediate. The conjugation of glutathione to LTA4 results in the formation of LTC4. LTC4 is rapidly metabolized to LTD4 and LTE4 as shown in Figure 1 (see page 6).3 This metabolism is essentially complete within 10 minutes in the human lung. LTC4, LTD4, and LTE4 are collectively referred to as cysteinyl leukotrienes (CysLTs).

LTC4 and LTD4 are potent mediators of asthma and hypersensitivity. They induce bronchoconstriction, increase microvascular permeability, and are vasoconstrictors of coronary arteries.4 The biological activity of LTE4 is much lower in most systems studied, but its presence reflects the prior existence of LTC4 and LTD4.

About This Assay

Cayman’s CysLT EIA Kit is a competitive assay that can be used for quantification of CysLT in urine and other sample matrices. The EIA typically displays an IC50 (50% B/B0) of approximately 103 pg/ml and a detection limit (80% B/B0) of approximately 34 pg/ml.
This assay is based on the competition between CysLTs and a CysLT-acetylcholinesterase (AChE) conjugate (CysLT Tracer) for a limited amount of CysLT EIA Monoclonal Antibody. Because the concentration of the CysLT Tracer is held constant while the concentration of CysLT varies, the amount of CysLT Tracer that is able to bind to the CysLT Monoclonal Antibody will be inversely proportional to the concentration of CysLT in the well. This antibody-CysLT complex binds to a goat polyclonal anti-mouse 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 CysLT Tracer bound to the well, which is inversely proportional to the amount of free CysLT present in the well during the incubation; or

\[
\text{Absorbance } \propto \frac{[\text{Bound CysLT Tracer}]}{[\text{CysLT}]} = 1/[\text{CysLT}]
\]

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

**Figure 1. The formation of CysLTs**

**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 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 9). 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 (ε = 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](image-url)
**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.

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

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

**Standard Curve**: a plot of the %B/B₀ 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. **Immunoassay Buffer A 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. **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 Concentrate (400X) (96-well kit; Item No. 400062)**: Dilute to a total volume of 2 liters with UltraPure Water and add 1 ml of Tween 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 Tween 20 (Item No. 400035).

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

   **NOTE**: Tween 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 demonstrated to work with urine. 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.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.
- Samples of mouse or rat origin may contain antibodies which interfere with the assay by binding to the goat anti-mouse plate. We recommend that all murine samples be purified prior to use in this assay.
- Samples containing BSA or FBS may interfere with this assay.

NOTE: It is essential that all samples and standards be diluted with UltraPure Water not Immunoassay Buffer A.

Testing for Interference

Plasma, serum, and whole blood, as well as other heterogeneous mixtures such as lavage fluid and aspirates, often contain contaminants which can interfere in immunoassays. 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 obtain at least two different dilutions of each sample between approximately 35 and 350 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 CysLT concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised.

Urine

CysLTs are excreted in urine as intact LTE₄ (~9-12%) and LTE₄ metabolites. Since LTC₄ and LTD₄ are virtually absent from urine, CysLT measurements in urine samples can be accomplished by measuring LTE₄ specifically (LTE₄ EIA Kit, Item No. 520411; please note urine samples measured with this kit will require purification). The levels of LTE₄ reported in urine vary depending on the method of measurement. Values obtained from HPLC (50-80 pg/mg creatinine), mass spectrometry (25-40 pg/mg creatinine), or HPLC followed by EIA (20-160 pg/mg creatinine) are generally lower than values from EIA measurements alone (200-1,500 pg/mg creatinine), suggesting the presence of cross-reactive compounds in unpurified urine. Interference in urine is infrequent; dilutions appropriate for this assay (i.e., dilutions falling between 20-80% B/B₀) show a direct linear correlation between CysLT immunoreactivity and CysLT concentration (see Figure 4, on page 14). These data suggest dilution in water and measurement in EIA is appropriate. As with any urinary marker, we recommend standardizing the values obtained by EIA to creatinine levels (Item No. 500701).
CysLT spiked into urine (pg/ml)

Slope = 1.047
y-intercept = 81.4 pg/ml
r² = 0.999

Figure 4. Recovery of CysLT from urine
Urine samples were spiked with CysLT, diluted as described in the Sample Preparation section and analyzed using the CysLT EIA Kit. The y-intercept corresponds to the amount of CysLT in unspiked urine. Error bars represent standard deviations obtained from multiple dilutions of each sample.

<table>
<thead>
<tr>
<th>Level</th>
<th>%CV Intra-assay variation</th>
<th>Average (pg/ml)</th>
<th>%CV Inter-assay variation</th>
<th>Average (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>6.6</td>
<td>520</td>
<td>12.5</td>
<td>869</td>
</tr>
<tr>
<td>Medium</td>
<td>10</td>
<td>198</td>
<td>14.3</td>
<td>356</td>
</tr>
<tr>
<td>Low</td>
<td>9.2</td>
<td>95</td>
<td>12.6</td>
<td>121</td>
</tr>
</tbody>
</table>

Table 1. Urine sample validation
Urine samples containing a high, medium, or low level of CysLT were measured 60 times each using a single set of reagents. The calculated percent CV is reported as intra-assay variance. A separate series of urine samples containing a high, medium, or low level of CysLTs were measured four times each using eight independent sets of reagents. The calculated percent CV is reported as inter-assay variance.

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). Due to the complex nature of these samples there may be significant interference in unpurified samples. Albumin or other proteins in the sample are likely to interfere with the optimal performance of this assay. For this reason, we recommend testing for interference before using these samples directly in the immunoassay.

Plasma
Plasma samples should be collected in vacutainers containing sodium heparin, EDTA, or sodium citrate. Plasma is a complex matrix that contains many substances that can interfere with this assay.

Culture Medium
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). Culture medium may contain substances that can interfere with this assay. We recommend testing for interference before using these samples directly in the immunoassay.
Preparation of Assay-Specific Reagents

Cysteinyl Leukotriene 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 CysLT EIA Standard (Item No. 420504) 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: It is essential that all samples and standards be diluted with UltraPure Water not Immunoassay Buffer A.

To prepare the standard for use in EIA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl UltraPure Water to tube #1 and 500 µl UltraPure Water 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 two hours.

Cysteinyl Leukotriene AChE Tracer

Reconstitute the CysLT AChE Tracer as follows:

100 dtn CysLT AChE Tracer (96-well kit; Item No. 400390): Reconstitute with 6 ml Immunoassay Buffer A.

OR

500 dtn CysLT AChE Tracer (480-well kit; Item No. 400390): Reconstitute with 30 ml Immunoassay Buffer A.

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

NOTE: Do not store tracer with dye for more than 24 hours.

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.

Cysteinyl Leukotriene EIA Monoclonal Antibody

Reconstitute the CysLT EIA Monoclonal Antibody as follows:

100 dtn CysLT EIA Monoclonal Antibody (96-well kit; Item No. 400392): Reconstitute with 6 ml Immunoassay Buffer A.

OR

500 dtn CysLT EIA Monoclonal Antibody (480-well kit; Item No. 400392): Reconstitute with 30 ml Immunoassay Buffer A.

Store the reconstituted CysLT EIA Monoclonal Antibody at 4°C. It will be stable for at least four weeks. A 20% surplus of antibody 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). NOTE: Do not store antibody 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 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 22, for more details). We suggest you record the contents of each well on the template sheet provided (see page 31).

<table>
<thead>
<tr>
<th>Plate Format</th>
<th>Blk - Blank</th>
<th>TA - Total Activity</th>
<th>NSB - Non-Specific Binding</th>
<th>B0 - Maximum Binding</th>
<th>S1-S8 - Standards 1-8</th>
<th>1-24 - Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Blk S1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1-9</td>
<td>1-9</td>
</tr>
<tr>
<td>B</td>
<td>Blk S2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>10-18</td>
<td>10-18</td>
</tr>
<tr>
<td>C</td>
<td>NSB S3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>11-19</td>
<td>11-19</td>
</tr>
<tr>
<td>D</td>
<td>NSB S4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>12-20</td>
<td>12-20</td>
</tr>
<tr>
<td>E</td>
<td>B0 S5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>13-21</td>
<td>13-21</td>
</tr>
<tr>
<td>F</td>
<td>B0 S6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>14-22</td>
<td>14-22</td>
</tr>
<tr>
<td>G</td>
<td>B0 S7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>15-23</td>
<td>15-23</td>
</tr>
<tr>
<td>H</td>
<td>TA S8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>16-24</td>
<td>16-24</td>
</tr>
</tbody>
</table>

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. UltraPure Water
   Add 50 µl UltraPure Water to Non-Specific Binding (NSB) wells. Add 50 µl UltraPure Water to Maximum Binding (B0) wells.

2. Immunoassay Buffer A
   Add 50 µl Immunoassay Buffer A to Non-Specific Binding (NSB) wells.

3. CysLT 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.

4. 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).

5. CysLT AChE Tracer
   Add 50 µl to each well except the Total Activity (TA) and the Blank (Blk) wells.

6. CysLT EIA Monoclonal Antibody
   Add 50 µl to each well except the Total Activity (TA), the Non-Specific Binding (NSB), and the Blank (Blk) wells within 15 minutes of addition of the tracer.
### Table 2. 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 be 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. Dilute 10 µl of Tracer into 90 µl Immunoassay Buffer A. Add 5 µl of the 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., B₀ wells ≥0.3 A.U. (blank subtracted)) in 90-120 minutes.

#### Reading the Plate
1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the 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 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.
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/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/eia) 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 % Bound. This value should approximate the % Bound that can be calculated from the Sample Data (see page 24). 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 28 for Troubleshooting).

Plot the Standard Curve

Plot %B/B₀ for standards S1-S8 versus CysLT 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.

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

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

Determine the Sample Concentration

Calculate the B/B₀ (or %B/B₀) value for each sample. Determine the concentration of each sample by identifying the %B/B₀ on the standard curve and reading the corresponding values on the x-axis. NOTE: Remember to account for any 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.

Recovery

The original concentration of the sample and recovery factor can be determined by the following method:

\[ V = \text{EIA determined concentration of the unspiked sample (pg/ml)} \]

\[ S = \text{concentration of the spike (pg/ml)} \]

\[ Y = \text{EIA determined concentration of spiked sample (pg/ml)} \]

\[ \text{CysLT (pg) in purified sample} = \frac{V}{\text{Recovery Factor}} \times \text{reconstituted volume of sample} \]

\[ \text{CysLT (pg) in original sample (pg/ml)} = \frac{\text{CysLT (pg) in purified sample}}{\text{Volume of sample used for purification (ml)}} \]

\[ \text{Purification Recovery Factor} = \left( \frac{Y - V}{S} \right) \]
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.

<table>
<thead>
<tr>
<th>Total Activity</th>
<th>Raw Data</th>
<th>Average</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/B₀</td>
<td>1.13</td>
<td>1.095</td>
<td></td>
</tr>
<tr>
<td>NSB</td>
<td>0.018</td>
<td>0.019</td>
<td>0.019</td>
</tr>
<tr>
<td>B₀</td>
<td>1.138</td>
<td>1.144</td>
<td>1.114</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose (pg/ml)</th>
<th>Raw Data</th>
<th>Corrected</th>
<th>%B/B₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>0.102</td>
<td>0.083</td>
<td>7.3</td>
</tr>
<tr>
<td>500</td>
<td>0.147</td>
<td>0.128</td>
<td>11.3</td>
</tr>
<tr>
<td>250</td>
<td>0.278</td>
<td>0.259</td>
<td>22.9</td>
</tr>
<tr>
<td>125</td>
<td>0.519</td>
<td>0.500</td>
<td>44.1</td>
</tr>
<tr>
<td>62.5</td>
<td>0.756</td>
<td>0.737</td>
<td>65.0</td>
</tr>
<tr>
<td>31.3</td>
<td>0.925</td>
<td>0.906</td>
<td>80.0</td>
</tr>
<tr>
<td>15.6</td>
<td>1.031</td>
<td>1.012</td>
<td>89.3</td>
</tr>
<tr>
<td>7.8</td>
<td>1.107</td>
<td>1.088</td>
<td>96.0</td>
</tr>
</tbody>
</table>

Table 3. Typical results

50% B/B₀ - 103 pg/ml
Detection Limit (80% B/B₀) - 34 pg/ml

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 25 and in the table below.

<table>
<thead>
<tr>
<th>Dose (pg/ml)</th>
<th>%CV* Intra-assay variation</th>
<th>%CV* Inter-assay variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>13.9</td>
<td>11.7</td>
</tr>
<tr>
<td>500</td>
<td>5.7</td>
<td>8.4</td>
</tr>
<tr>
<td>250</td>
<td>4.7</td>
<td>7.8</td>
</tr>
<tr>
<td>125</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td>62.5</td>
<td>6.9</td>
<td>6.6</td>
</tr>
<tr>
<td>31.3</td>
<td>9.4</td>
<td>14.3</td>
</tr>
<tr>
<td>15.6</td>
<td>19.4</td>
<td>†</td>
</tr>
<tr>
<td>7.8</td>
<td>†</td>
<td>†</td>
</tr>
</tbody>
</table>

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
†Outside of the recommended usable range of the assay.

**Specificity:**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukotriene C₄</td>
<td>100%</td>
</tr>
<tr>
<td>Leukotriene D₄</td>
<td>100%</td>
</tr>
<tr>
<td>Leukotriene E₄</td>
<td>79%</td>
</tr>
<tr>
<td>5,6-DiHETE</td>
<td>3.7%</td>
</tr>
<tr>
<td>Leukotriene B₄</td>
<td>1.3%</td>
</tr>
<tr>
<td>5(S)-HETE</td>
<td>0.04%</td>
</tr>
<tr>
<td>Arachidonic Acid</td>
<td>&lt;0.01%</td>
</tr>
</tbody>
</table>

Table 5. Specificity of the CysLT EIA Monoclonal Antibody
## Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erratic values; dispersion of duplicates</td>
<td>A. Trace organic contaminants in the water source</td>
<td>Replace activated carbon filter or change source of UltraPure Water</td>
</tr>
<tr>
<td></td>
<td>B. Poor pipetting/technique</td>
<td></td>
</tr>
<tr>
<td>High NSB (&gt;0.075)</td>
<td>A. Poor washing</td>
<td>Revase plate and redevelop</td>
</tr>
<tr>
<td></td>
<td>B. Exposure of NSB wells to specific antibody</td>
<td></td>
</tr>
<tr>
<td>Very low B0</td>
<td>A. Trace organic contaminants in the water source</td>
<td>Replace activated carbon filter or change source of UltraPure Water</td>
</tr>
<tr>
<td></td>
<td>B. Plate requires additional development time</td>
<td>Return plate to shaker and re-read later</td>
</tr>
<tr>
<td></td>
<td>C. Dilution error in preparing reagents</td>
<td></td>
</tr>
<tr>
<td>Low sensitivity (shift in dose response curve)</td>
<td>Standard is degraded</td>
<td>Replace standard</td>
</tr>
<tr>
<td>Analyses of two dilutions of a biological sample do not agree</td>
<td>Interfering substances are present</td>
<td>Purify sample prior to analysis by EIA&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>(i.e., more than 20% difference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only Total Activity (TA) wells develop</td>
<td>Trace organic contaminants in the water source</td>
<td>Replace activated carbon filter or change source of UltraPure Water</td>
</tr>
</tbody>
</table>

## References

Related Products

- Cysteinyl Leukotriene Express EIA Kit - Item No. 10009291
- Leukotriene C₄ EIA Kit - Item No. 520211
- Leukotriene E₄ EIA Kit - Item No. 520411
- SPE Cartridges (C-18) - Item No. 400020
- UltraPure Water - Item No. 400000

Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer’s exclusive remedy and Cayman’s sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman’s option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.