Leukotriene B₄ Express ELISA Kit

Item No. 10009292
# GENERAL INFORMATION

## Materials Supplied

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item Description</th>
<th>Quantity/Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>10009434</td>
<td>Leukotriene B&lt;sub&gt;4&lt;/sub&gt; Express ELISA Monoclonal Antibody</td>
<td>1 vial/100 dtn/1 vial/500 dtn</td>
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<tr>
<td>10009435</td>
<td>Leukotriene B&lt;sub&gt;4&lt;/sub&gt; Express AChE Tracer</td>
<td>1 vial/100 dtn/1 vial/500 dtn</td>
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<td>10009436</td>
<td>Leukotriene B&lt;sub&gt;4&lt;/sub&gt; Express ELISA Standard</td>
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<td>10011325</td>
<td>Immunoassay Buffer A Concentrate (10X)</td>
<td>2 vials/10 ml/4 vials/10 ml</td>
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<tr>
<td>400062</td>
<td>Wash Buffer Concentrate (400X)</td>
<td>1 vial/5 ml/1 vial/12.5 ml</td>
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<td>400035</td>
<td>Polysorbate 20</td>
<td>1 vial/3 ml/1 vial/3.5 ml</td>
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<td>400008</td>
<td>Goat Anti-Mouse IgG Coated Plate</td>
<td>1 plate/5 plates</td>
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<tr>
<td>400012</td>
<td>96-Well Cover Sheet</td>
<td>1 cover/5 covers</td>
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<tr>
<td>400050</td>
<td>Ellman's Reagent</td>
<td>3 vials/100 dtn/6 vials/250 dtn</td>
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<tr>
<td>400040</td>
<td>ELISA Tracer Dye</td>
<td>1 vial</td>
</tr>
<tr>
<td>400042</td>
<td>ELISA Antiserum Dye</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) 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 B<sub>4</sub> 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 at -20°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).
4. Materials used for Sample Preparation (see page 12).
Background

Leukotriene B4 (LTB₄) is synthesized from arachidonic acid by the combined action of 5-lipoxygenase and LTA₄ hydrolase.¹ LTB₄ has long been recognized as a potent mediator of inflammation. It stimulates a number of leukocyte functions, including aggregation, stimulation of ion fluxes, enhancement of lysosomal enzyme release, superoxide anion production, chemotaxis, and chemokinesis.²⁻³ In subnanomolar ranges (3.9 x 10⁻¹⁰ M), LTB₄ causes chemotaxis and chemokinesis in human neutrophils.² At higher concentrations, (1.0 x 10⁻⁷ M), LTB₄ leads to neutrophil aggregation and degranulation as well as superoxide anion production.²⁻⁴ Plasma levels of LTB₄ increase from <100 pg/ml to >100 ng/ml following leukocyte stimulation.⁵⁻⁷ LTB₄ is metabolized in leukocytes and hepatocytes to less active 20-hydroxy and 20-carboxy LTB₄ by NADPH-dependent cytochrome P450 enzymes followed by β-oxidation at the ω-end to ω-carboxy dinor LTB₄ and ω-carboxy tetranor-LTB₃.³⁻⁸⁻¹⁰ LTB₄ is not excreted in the urine.¹¹

About This Assay

Cayman’s LTB₄ Express ELISA Kit is a competitive assay that can be used for quantification of LTB₄ in plasma and other sample matrices. The assay has a range from 15.6-2,000 pg/ml and a sensitivity (80% B/B₀) of approximately 45 pg/ml.

Description of AChE Competitive ELISAs¹²⁻¹⁴

This assay is based on the competition between LTB₄ and an LTB₄-acetylcholinesterase (AChE) conjugate (LTB₄ Tracer) for a limited amount of LTB₄ monoclonal antibody binding sites. Because the concentration of the LTB₄ Tracer is held constant while the concentration of LTB₄ varies, the amount of LTB₄ Tracer that is able to bind to the LTB₄ monoclonal antibody will be inversely proportional to the concentration of LTB₄ in the well. This antibody-LTB₄ 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 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 LTB₄ Tracer bound to the well, which is inversely proportional to the amount of free LTB₄ present in the well during the incubation; or

\[
\text{Absorbance} \propto \frac{\text{Bound LTB}_4 \text{ Tracer}}{\text{LTB}_4} \propto \frac{1}{\text{LTB}_4}
\]

A schematic of this process is shown below in Figure 1.

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**Figure 1. Schematic of the AChE ELISA**

- Plates are pre-coated with goat anti-mouse IgG and blocked with a proprietary formulation of proteins.
- Incubate with tracer, antibody, and either standard or sample.
- Wash to remove all unbound reagents.
- Develop the well with Ellman’s Reagent.

- Goat Anti-Mouse IgG
- Blocking proteins
- AChE linked to LTB₄ (tracer)
- Specific antibody to LTB₄
- Free LTB₄
Biochemistry of Acetylcholinesterase

The electric organ of the electric eel, *E. electricus*, contains an avid 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 AChE 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 2, 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 redevelopment 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 2. Reaction catalyzed by acetylcholinesterase
Definition of Key Terms

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

Total Activity: total enzymatic activity of the AChE-linked tracer. This is analogous to the specific activity of a radioactive tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding. Do not forget to subtract the Blank absorbance values.

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.

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/B0) value of the tested molecule to the mid-point (50% B/B0) value of the primary analyte when each is measured in assay buffer using the following formula:

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

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. Immunoassay Buffer A Concentrate (10X)
   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 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 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.

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).
Sample Preparation

In general tissue culture supernatant samples may be diluted with ELISA Buffer and added directly to the assay well. Plasma, serum, whole blood, as well as other heterogeneous mixtures such as CSF often contain contaminants which can interfere in the assay. It is best to check for interference before beginning 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 50 and 500 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 LTB₄ concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. The Purification Protocol below is one such method.

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 origin may contain antibodies which interfere with the assay by binding to the goat anti-mouse plate. We recommend that all mouse samples be purified prior to use in this assay.

Plasma

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate. Indomethacin should be added immediately after whole blood collection (sufficient to give a 10 µM final concentration). Indomethacin will prevent ex vivo formation of prostaglandins and thromboxanes, which have the potential to interfere with this assay. Centrifuge the whole blood at 1,500 x g at 4°C for 20 minutes and collect the plasma. The plasma sample can be used immediately or stored at -80°C.

Processing Plasma

To the plasma sample add two volumes of ice-cold acetone and vortex briefly. Incubate for 15 minutes on ice and centrifuge at 15,000 x g for five minutes. Transfer the supernatant (containing the LTB₄) to a clean test tube and evaporate the acetone under a stream of nitrogen. Reconstitute the dried material with a volume of Immunoassay Buffer A equal to the original plasma volume. If you wish to concentrate your samples, reconstitute in a volume of Immunoassay Buffer A that is smaller than the original plasma volume. The reconstituted samples could be used immediately.

Figure 3. Recovery of Leukotriene B₄ from Plasma

Plasma samples were spiked with LTB₄ and processed as described above. The y-intercept corresponds to the amount of LTB₄ in unspiked plasma. Error bars represent standard deviations obtained from multiple dilutions of each sample.
Preparation of Assay-Specific Reagents

Leukotriene B\textsubscript{4} Express ELISA Standard

Equilibrate a pipette tip in ethanol by repeatedly filling and expelling the tip with ethanol. Using the equilibrated pipette tip, transfer 100 µl of the Leukotriene B\textsubscript{4} Express ELISA Standard (Item No. 10009436) into a clean test tube, then dilute with 900 µl of UltraPure water. The concentration of this solution (the bulk standard) will be 20 ng/ml. Store this solution at 4°C; it will be stable for approximately four weeks.

To prepare the standard for use in the ELISA: Obtain eight clean test tubes and number them #1-8. Aliquot 900 µl of Immunoassay Buffer A to tube #1 and 500 µl of Immunoassay Buffer A to tubes #2-8. Transfer 100 µl of the bulk standard (20 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 into tube #3; mix thoroughly. Repeat this process for tubes #4-8. The diluted standards may be stored at 4°C for no more than 24 hours.

Figure 4. Preparation of the Leukotriene B\textsubscript{4} standards

Leukotriene B\textsubscript{4} Express AChE Tracer

Reconstitute the LTB\textsubscript{4} Express AChE Tracer as follows:

100 dtn Leukotriene B\textsubscript{4} Express AChE Tracer (96-well kit; Item No. 10009435): Reconstitute with 6 ml Immunoassay Buffer A.

OR

500 dtn Leukotriene B\textsubscript{4} Express AChE Tracer (480-well kit; Item No. 10009435): Reconstitute with 30 ml Immunoassay Buffer A.

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

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer or add 300 µl of dye to 30 ml of tracer).

Leukotriene B\textsubscript{4} Express ELISA Monoclonal Antibody

Reconstitute the LTB\textsubscript{4} Express ELISA Monoclonal Antibody as follows:

100 dtn Leukotriene B\textsubscript{4} Express ELISA Monoclonal Antibody (96-well kit; Item No. 10009434): Reconstitute with 6 ml Immunoassay Buffer A.

OR

500 dtn Leukotriene B\textsubscript{4} Express ELISA Monoclonal Antibody (480-well kit; Item No. 10009434): Reconstitute with 30 ml Immunoassay Buffer A.

Store the reconstituted LTB\textsubscript{4} Express ELISA 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).
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 below in Figure 5. 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 20, for more details). We suggest you record the contents of each well on the template sheet provided (see page 30).

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
   
   Add 100 µl Immunoassay Buffer A to NSB wells. Add 50 µl Immunoassay Buffer A to B₀ wells.

2. Leukotriene B₄ 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
   
   Dilute samples a minimum of 1:2 with Immunoassay Buffer A. 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 B₄ Express AChE Tracer
   
   Add 50 µl to each well except the TA and the Blk wells.

5. Leukotriene B₄ Express ELISA Monoclonal Antibody
   
   Add 50 µl to each well except the TA, the NSB, and the Blk wells.
### Table 1. Pipetting summary

<table>
<thead>
<tr>
<th>Well</th>
<th>Immunoassay Buffer A</th>
<th>Standard/ Sample</th>
<th>Tracer</th>
<th>Antibody</th>
</tr>
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<tbody>
<tr>
<td>Blk</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TA</td>
<td>-</td>
<td>-</td>
<td>5 µl (at devl. step)</td>
<td>-</td>
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<tr>
<td>NSB</td>
<td>100 µl</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
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<tr>
<td>(B_0)</td>
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<td>-</td>
<td>50 µl</td>
<td>50 µl</td>
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<tr>
<td>Std/Sample</td>
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<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
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</table>

### Incubation of the Plate

Cover each plate with plastic film (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 dtw vial Ellman’s Reagent (96-well kit; Item No. 400050):** Reconstitute with 20 ml of UltraPure water.

   **OR**

   **250 dtw vial Ellman’s Reagent (480-well kit; Item No. 400050):** Reconstitute with 50 ml of UltraPure water.

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

2. Empty the wells and rinse five times with Wash Buffer.
3. Add 200 µl of Ellman’s Reagent to each well.
4. Dilute the LTB\(_4\) Express AChE Tracer 1:10 with Immunoassay Buffer A (for example, 50 µl Tracer into 450 µl Assay Buffer).
5. Add 5 µl of tracer to the TA wells.
6. Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (i.e., \(B_0\) wells ≥0.3 A.U. (blank subtracted)) in 60-90 minutes.

### Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the plate cover being careful to keep Ellman’s Reagent from splashing on the cover. **NOTE:** Any loss of Ellman’s Reagent will affect the absorbance readings. If Ellman’s Reagent is present on the cover, use a pipette to transfer the Ellman’s Reagent into the well. If too much Ellman’s Reagent has splashed on the cover to easily redistribute back into the wells, wash the plate three times with wash buffer and repeat the development with fresh Ellman’s Reagent.
3. Read the plate at a wavelength between 405 and 420 nm. The absorbance may be checked periodically until the \(B_0\) 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 0.3-1.0 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.
Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as either %B/B₀ versus log concentration using a four-parameter logistic fit or as logit B/B₀ versus log concentration using a linear fit. NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/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 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 TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool; the corrected B₀ divided by the actual TA (10X measured absorbance) will give the %Bound. This value should closely approximate the %Bound that can be calculated from the Sample Data (see page 22). 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 27 for Troubleshooting).

Plot the Standard Curve

Plot %B/B₀ for standards S1-S8 versus LTB₄ 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 using the equation obtained from the standard curve plot. NOTE: Remember to account for any concentration or dilution of the sample prior to the addition to the well. Samples with %B/B₀ values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.
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>Dose (pg/ml)</th>
<th>Raw Data</th>
<th>Corrected</th>
<th>%B/B₀</th>
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<td>2,000</td>
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<td>0.146</td>
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<td>500</td>
<td>0.292</td>
<td>0.287</td>
<td>20.8</td>
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Table 2. Typical results

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<th>Average</th>
<th>Corrected</th>
<th>%B/B₀</th>
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<td>0.659</td>
<td>0.004</td>
<td>0.006</td>
</tr>
<tr>
<td>1.392</td>
<td>1.409</td>
<td>1.406</td>
<td>1.409</td>
<td>1.406</td>
</tr>
</tbody>
</table>

Figure 8. Typical standard curve

Assay Range = 15.6-2,000 pg/ml
Sensitivity (defined as 80% B/B₀) = 45 pg/ml
Mid-point (defined as 50% B/B₀) = 100-200 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with Assay Buffer.
Precision:
The intra-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 23 and in the table below.

<table>
<thead>
<tr>
<th>Dose (pg/ml)</th>
<th>%CV* Intra-assay variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,000</td>
<td>8.6</td>
</tr>
<tr>
<td>1,000</td>
<td>6.5</td>
</tr>
<tr>
<td>500</td>
<td>3.9</td>
</tr>
<tr>
<td>250</td>
<td>3.9</td>
</tr>
<tr>
<td>125</td>
<td>7.8</td>
</tr>
<tr>
<td>62.5</td>
<td>8.0</td>
</tr>
<tr>
<td>31.3</td>
<td>11.1</td>
</tr>
<tr>
<td>15.6</td>
<td>19.9</td>
</tr>
</tbody>
</table>

Table 3. Plasma sample validation
Plasma samples containing a high, medium, or low level of LTB₄ were measured 60 times each using a single set of reagents. The calculated %CV is reported as intra-assay variance. A separate series of plasma samples containing a high, medium, or low level of LTB₄ were measured four times each using eight independent sets of reagents. The calculated %CV is reported as inter-assay variance.

<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>7.7</td>
<td>689</td>
<td>10.9</td>
<td>904</td>
</tr>
<tr>
<td>Medium</td>
<td>6.6</td>
<td>427</td>
<td>11.7</td>
<td>575</td>
</tr>
<tr>
<td>Low</td>
<td>8.0</td>
<td>260</td>
<td>9.2</td>
<td>367</td>
</tr>
</tbody>
</table>

Table 3. Intra- and inter-assay variation
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
†Outside of the recommended usable range of the assay.
Cross Reactivity:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross Reactivity</th>
<th>Compound</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukotriene B₄</td>
<td>100%</td>
<td>15(S)-HETE</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>20-hydroxy Leukotriene B₄</td>
<td>260%</td>
<td>Leukotriene C₄</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Leukotriene B₅*</td>
<td>44%</td>
<td>Leukotriene D₄</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>20-carboxy Leukotriene B₄</td>
<td>13.4%</td>
<td>Leukotriene E₄</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>6-trans Leukotriene B₄</td>
<td>0.16%</td>
<td>Resolvin D1</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>6-trans-12-epi Leukotriene B₄</td>
<td>0.12%</td>
<td>Resolvin D2</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>5(S)-HETE</td>
<td>0.01%</td>
<td>12(S)-HETE</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Arachidonic Acid</td>
<td>&lt;0.01%</td>
<td>Arachidonyl Ethanolamide</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>5,6-DiHETE</td>
<td>&lt;0.01%</td>
<td>Prostaglandin E₂</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>5(R)-HETE</td>
<td>&lt;0.01%</td>
<td>Prostaglandin F₂₂x</td>
<td>&lt;0.01%</td>
</tr>
</tbody>
</table>

Table 4. Cross Reactivity of the Leukotriene B₄ Express ELISA

*Leukotriene B₅, derived from n-3 fatty acids, is mostly detectable in humans and animals with a diet rich in, or supplemented with, eicosapentaenoic acid.¹⁵

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>A. 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;10% of B₀)</td>
<td>A. Poor washing</td>
<td>A. Rewash 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>A. Replace activated carbon filter or change source of UltraPure water</td>
</tr>
<tr>
<td></td>
<td>B. Plate requires additional development time</td>
<td></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 (i.e., more than 20% difference)</td>
<td>Interfering substances are present</td>
<td>Purify sample prior to analysis by ELISA¹⁶</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


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