Lipoxin B_4 ELISA Kit

Item No. 501920

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	96 wells Quantity/Size	Storage Temperature
401920	Lipoxin B_4-AChE Tracer	1 vial/100 dtn	-20°C
401922	Lipoxin B_4 ELISA Polyclonal Antiserum	1 vial/100 dtn	-20°C
401924	Lipoxin B_4 ELISA Standard	1 vial	-80°C
400060	ELISA Buffer Concentrate (10X)	1 vial/10 ml	Room Temperature
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	Room Temperature
400035	Polysorbate 20	1 vial/3 ml	Room Temperature
400004/400006	Mouse Anti-Rabbit IgG-Coated Plate	1 plate	4°C
400050	Ellman's Reagent	3 vials/100 dtn	-20°C
400040	ELISA Tracer Dye	1 ea	Room Temperature
400042	ELISA Antiserum Dye	1 ea	Room Temperature
400012	96-Well Cover Sheet	1 ea	Room Temperature

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.

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 in the Materials Supplied section (see page 3) 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 at 414 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of ultrapure water is recommended. Pure water - glass distilled or deionized - may not be acceptable. *NOTE: Ultrapure water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 12)

INTRODUCTION

Background

Lipoxin B_4 (LXB_4) is a part of the specialized pro-resolving mediator (SPM) family of polyunsaturated fatty acid (PUFA) metabolites and a positional isomer of LXA_4.[1,2] LXB_4 is formed from arachidonic acid through double lipoxygenase-catalyzed reactions initiated by either 5-lipoxygenase (5-LO) followed by 12-LO/15-LO, with a leukotriene A_4 (LTA_4) intermediate, or by 15-LO followed by 5-LO, with 15(S)-HETE and 5(S)-Hp-15(S)-HETE intermediates.[1,3,4] The generation of LXB_4 typically requires transcellular metabolism of arachidonic acid.[1,2] For example, LTA_4 that is synthesized in neutrophils by 5-LO is metabolized to LXB_4 in platelets by 12-LO.[1]

LXB_4 is generated during the resolution phase of inflammation at the site of inflammation.[6] It inhibits LTB_4-induced polymorphonuclear (PMN) neutrophil migration and P-selectin-mediated neutrophil-endothelial cell adhesion *in vitro*, and also stimulates non-phlogistic phagocytosis of apoptotic neutrophils by macrophages.[5,7] LXB_4 reduces mast cell degranulation, as well as airway inflammation and hyper-responsiveness in a mouse model of allergic rhinitis.[8] It also reduces edema and has anti-hyperalgesic effects in a mouse model of inflammatory pain.[9]

About This Assay

Cayman's LXB_4 ELISA Kit is a competitive assay that can be used for quantification of LXB_4 in plasma, serum, and urine. The assay has a range of 1.6-1,000 pg/ml with a midpoint (50% B/B_0) of approximately 41 pg/ml and a sensitivity (80% B/B_0) of approximately 9.5 pg/ml.

Principle Of This Assay

This assay is based on the competition between native LXB_4 and an LXB_4-acetylcholinesterase (AChE) conjugate (LXB_4-AChE Tracer) for a limited amount of LXB_4 Polyclonal Antiserum. Because the concentration of the LXB_4-AChE Tracer is held constant while the concentration of native LXB_4 varies, the amount of LXB_4-AChE Tracer that is able to bind to the LXB_4 Polyclonal Antiserum will be inversely proportional to the concentration of native LXB_4 in the well. This antibody-LXB_4 complex binds to mouse anti-rabbit 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 at 414 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of LXB_4-AChE Tracer bound to the well, which is inversely proportional to the amount of free LXB_4 present in the well during the incubation, as described in the equation:

$$\text{Absorbance} \propto [\text{Bound } LXB_4\text{-AChE Tracer}] \propto 1/[LXB_4]$$

A schematic of this process is shown in Figure 1, on page 8.

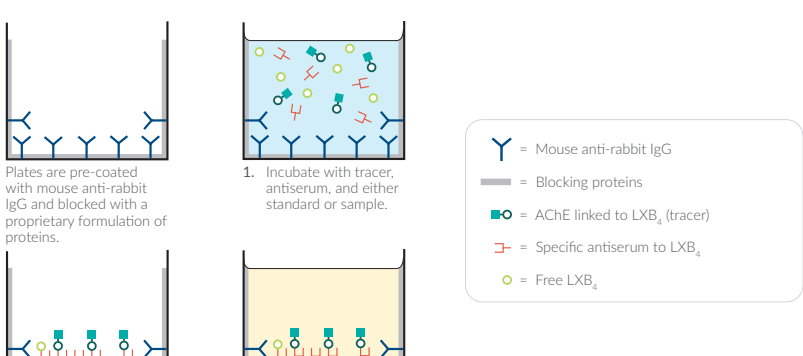


Figure 1. Schematic of the LXB_4 ELISA

Definition of Key Terms

Blank (Blk): 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 (TA): total enzymatic activity of the LXB_4-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antiserum 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 antiserum can bind in the absence of free analyte.

$\%B/B_0$ (%Bound/Maximum Bound): ratio of the absorbance of a particular sample or standard well to the average absorbance of the maximum binding (B_0) wells.

Standard Curve: a plot of the $\%B/B_0$ values *versus* concentration of a series of wells containing various known amounts of analyte.

Dtn: determination, 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 antiserum. 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:

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

Lower Limit of Detection (LLOD): 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 approximately two months.

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

   Dilute the contents of one vial of ELISA Buffer Concentrate (Item No. 400060) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.*

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 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 Polysorbate 20 to an end concentration of 0.5 ml/L. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water. 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

Testing for Interference

This assay has been validated using human plasma, serum, and urine. Other sample types should be tested for interference to evaluate the need for sample purification 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 100 pg/ml and 10 pg/ml (*i.e.*, between 30-80% B/B_0, which is 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 LXB_4 concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. Purification methods will need to be determined by the end user and tested for compatibility in the assay.

Plasma and Serum

It is recommended that plasma and serum samples be diluted at least 1:4 into ELISA Buffer (1X) prior to testing in the assay.

Urine

It is recommended that urine samples be diluted at least 1:2 into ELISA Buffer (1X) prior to testing in 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 rabbit origin may contain antibodies that interfere with the assay by binding to the mouse anti-rabbit plate. We recommend that all rabbit samples by purified prior to use in the assay.

Sample Matrix Properties

Linearity

To assess dilutional linearity, human plasma, serum, and urine samples were serially diluted with ELISA Buffer (1X) and evaluated for linearity using the LXB_4 ELISA Kit. The results are shown in the table below.

Dilution Factor	Concentration (pg/ml)	Dilutional Linearity (%)
Plasma		
4	65.18	100
8	71.13	109
16	75.10	115
Serum		
4	34.17	100
8	34.56	101
16	32.97	96.5
Urine		
2	21.99	100
4	20.31	92.4
8	20.56	93.5

Table 1. Dilutional linearity of human plasma, serum, and urine samples

Spike and Recovery

Human plasma, serum, and urine samples were spiked with different amounts of LXB_4, serially diluted with ELISA Buffer (1X) and analyzed using the LXB_4 ELISA Kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

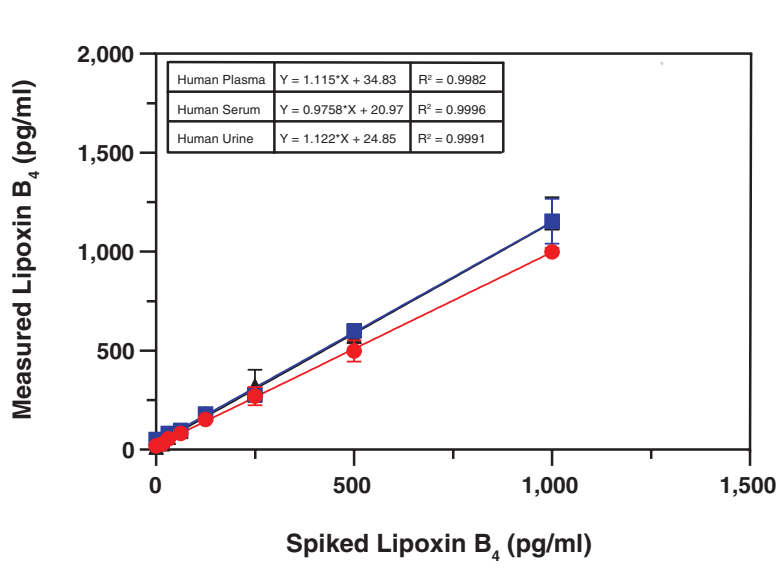


Figure 2. Spike and recovery in human plasma, serum, and urine samples

Parallelism

To assess parallelism, human plasma, serum, and urine samples were serially diluted and assayed at multiple dilutions using the LXB_4 ELISA Kit. Concentrations were plotted as a function of the sample dilution. The results are shown below.

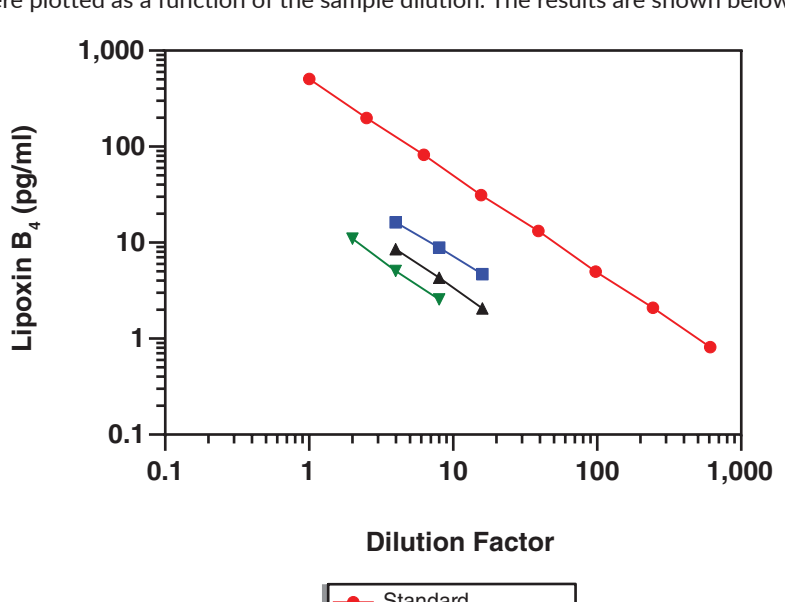


Figure 3. Parallelism of human plasma, serum, and urine samples

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Lipoxin B_4 ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the LXB_4 ELISA Standard (Item No. 401924) several times. Using the equilibrated pipette tip, transfer 100 µl of the standard into a clean test tube, then dilute with 400 µl ultrapure water. The concentration of this solution (the bulk standard) will be 10,000 pg/ml. Store it at 4°C and use within two weeks.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 900 µl ELISA Buffer (1X) to tube #1 and 600 µl ELISA Buffer (1X) to tubes #2-8. Transfer 100 µl of the bulk standard (10,000 pg/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 400 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 400 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than two hours.

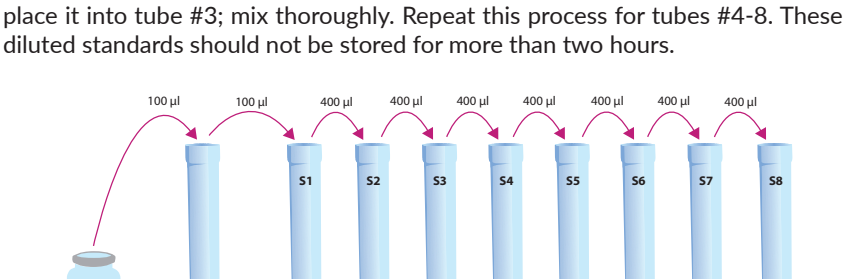


Figure 4. Preparation of the LXB_4 standards

Lipoxin B_4-AChE Tracer

Reconstitute the LXB_4-AChE Tracer (Item No. 401920) with 6 ml of ELISA Buffer (1X). Transfer the reconstituted tracer to a polypropylene tube or vial, store at 4°C (*do not freeze!*), and use within two weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer). *NOTE: Tracer with dye will be stable for 2 weeks if stored at 4°C.*

Lipoxin B_4 ELISA Polyclonal Antiserum

Reconstitute the LXB_4 ELISA Polyclonal Antiserum (Item No. 401922) with 6 ml of ELISA Buffer (1X). Store the reconstituted LXB_4 ELISA Polyclonal Antiserum at 4°C (*do not freeze!*) and use within 4 weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antiserum, if desired, to aid in visualization of antiserum-containing wells. Add the dye to the reconstituted antiserum at a final dilution of 1:100 (add 60 µl of dye to 6 ml antiserum). *NOTE: Antiserum with dye will be stable for 2 weeks if stored at 4°C.*

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 three 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 a minimum of 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 5, 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 recording the contents of each well on the template sheet provided (see page 33).

	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 5. Sample plate format

Performing the Assay

Pipetting Hints

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

Addition of the Reagents

1. **ELISA Buffer**

   Add 100 µl ELISA Buffer (1X) to NSB wells. Add 50 µl ELISA Buffer (1X) to B_0 wells.

2. **Lipoxin B_4 ELISA Standard**

   Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next 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 sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. **Lipoxin B_4-AChE Tracer**

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

5. **Lipoxin B_4 ELISA Polyclonal Antiserum**

   Add 50 µl to each well except the TA, NSB, and Blk wells within 15 minutes of addition of the tracer.

Incubation of the Plate

Cover each plate with a 96-Well Plate Cover Sheet (Item No. 400012) and incubate overnight at room temperature.

Development of the Plate

1. Reconstitute Ellman's Reagent (Item No. 400050) immediately before use with 20 ml of ultrapure water.
2. Empty the wells and rinse five times with ~300 µl 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 Plate Cover. 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 ≥0.4 A.U. (blank subtracted)) in 60 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.*
3. Read the plate at a wavelength of 414 nm.

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively, a spreadsheet program can be used. The data should be plotted as either %B/B_0 *versus* log concentration using a four-parameter logistic fit or as logit B/B_0 *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

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

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

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

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Erratic absorbance values could indicate the presence of organic solvents in the buffer or other technical problems (see page 31 for ***Troubleshooting****). Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system. Only the linear part of this standard curve should be used in the calculations.*

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* LXB_4 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 by identifying the %B/B_0 on the standard curve and reading the corresponding values on the x-axis. *NOTE: Remember to account for any dilution of the original sample concentration prior to its addition to the well. Samples with %B/B_0 values greater than 80% or less than 30% 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.*

NOTE: If there is an error in the B_0 wells it is possible to calculate sample concentrations by plotting the absorbance values and back calculating sample absorbance off 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 below to determine the values of your samples.

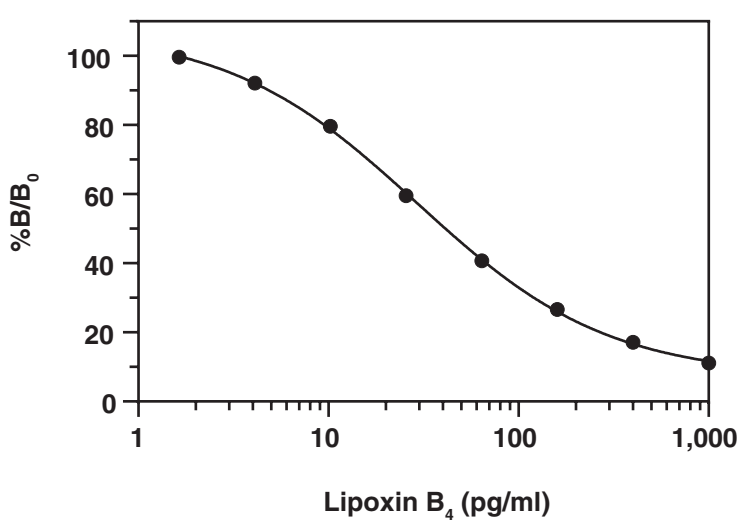
Absorbance at 414 nm (60 minutes)

Analyte Standards (pg/ml)	Blank-subtracted Absorbance	NSB-corrected Absorbance	%B/B_0	%CV* Intra-assay Precision	%CV* Inter-assay Precision
NSB	0.000				
B_0	0.643	0.643			
1,000	0.070	0.070	11.1	14.5	14.8
400	0.109	0.109	17.1	7.0	4.1
160	0.170	0.170	26.6	3.9	5.1
64	0.261	0.261	40.7	4.9	2.5
25.6	0.382	0.382	59.5	5.0	2.0
10.2	0.511	0.511	79.6	10.1	2.8
4.1	0.592	0.592	92.1	25.1**	8.5
1.6	0.640	0.640	99.6	32.8**	12.7
TA	0.232				

Table 2. Typical results

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve

**Evaluate data in this range with caution



Assay Range = 1.6-1,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 9.5 pg/ml
Mid-point (defined as 50% B/B_0) = 41.3 pg/ml
Lower Limit of Detection (LLOD) = 3.7 pg/ml

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

Figure 6. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three matrix controls (human urine) in a single assay.

Matrix Control (pg/ml)	%CV
1,387	13.6
797	9.5
215	8.3

Table 3. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of three matrix controls (human urine) in separate assays on different days.

Matrix Control (pg/ml)	%CV
1,513	6.8
808	6.2
187	8.6

Table 4. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity
Lipoxin B_4	100%
Lipoxin B_4 methyl ester*	54%
Lipoxin A_4	0.065%
15(S)-HETE	<0.01%
Leukotriene B_4	<0.01%
Prostaglandin E_2	<0.01%
15(S)-HpETE	<0.01%
Resolvin E_1	<0.01%
Arachidonic Acid	<0.01%

**Synthetic compound*

Table 5. Cross reactivity of the Lipoxin B_4 ELISA Kit

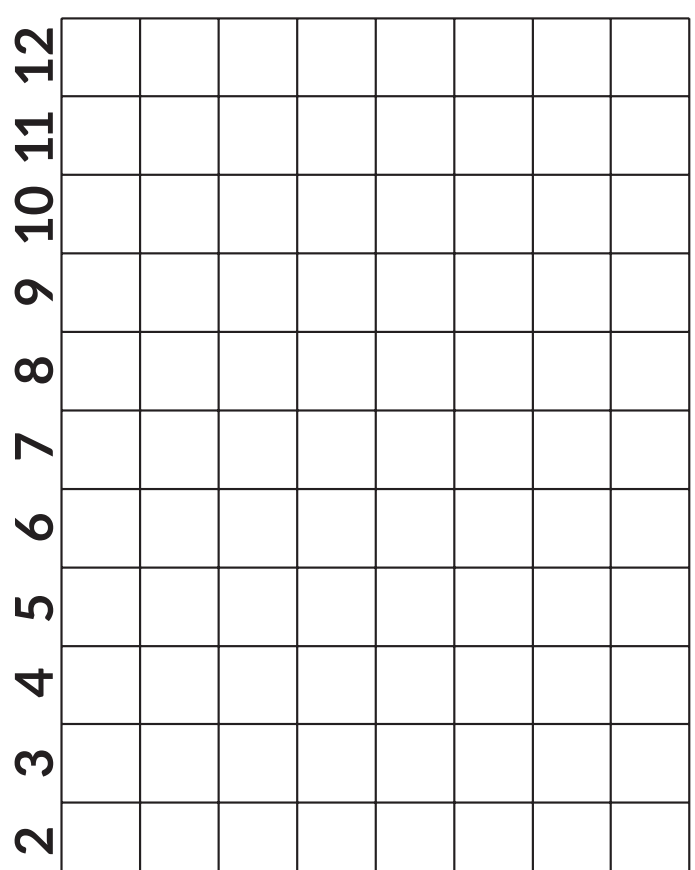
RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (>0.100 O.D.)	A. Poor washing; ensure proper washing is used B. Exposure of NSB wells to specific antibody
Very low B_0	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Low sensitivity (shift in dose-response curve)	Standard is degraded or contaminated
Analysis of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present; consider an alternative sample preparation

Procedure	Blk	TA	NSB	B_0	Standards/ Samples
Reconstitute and Mix	Mix all reagents gently				
ELISA Buffer (1X)	--	--	100 µl	50 µl	--
Standards/Samples	--	--	--	--	50 µl
LXB_4-AChE Tracer	--	--	50 µl	50 µl	50 µl
LXB_4 ELISA Polyclonal Antiserum	--	--	--	50 µl	50 µl
Seal	Seal the plate and tap gently to mix				
Incubate	Incubate plate overnight at room temperature				
Aspirate and Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)				
Apply Ellman's Reagent	200 µl	200 µl	200 µl	200 µl	200 µl
TA - Apply Tracer	--	5 µl	--	--	--
Seal	Seal plate and incubate for 60 minutes at room temperature on orbital shaker, protected from light				
Read	Read absorbance at 414 nm				

Table 6. Assay Summary



References

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NOTES

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