15(S)-HETE ELISA Kit

Item No. 534721

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TABLE OF CONTENTS

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

INTRODUCTION
6 Background
6 About this Assay
7 Principle Of This Assay
9 Definition of Key Terms

PRE-ASSAY PREPARATION
11 Buffer Preparation
12 Sample Preparation

ASSAY PROTOCOL
15 Preparation of Assay-Specific Reagents
18 Plate Set Up
20 Performing the Assay

ANALYSIS
23 Calculations
25 Performance Characteristics

RESOURCES
30 Troubleshooting
31 References
33 Plate Template
34 Notes
35 Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
434722	15(S)-HETE ELISA Antiserum	1 vial/100 dtn	1 vial/500 dtn
434720	15(S)-HETE-AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
434724	15(S)-HETE ELISA Standard	1 vial/0.5 ml	1 vial/0.5 ml
400060	ELISA Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	1 vial/12.5 ml
400035	Polysorbate 20	1 vial/3 ml	1 vial/3 ml
400005/400007	Mouse Anti-Rabbit IgG-Coated Plate	1 plate	5 plates
400012	96-Well Cover Sheet	1 ea	5 ea
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
400040	ELISA Tracer Dye	1 ea	1 ea
400042	ELISA Antiserum Dye	1 ea	1 ea

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 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. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 12)

INTRODUCTION

Background

15(S)-HETE is a hydroxy fatty acid formed *via* oxidation of arachidonic acid with a 15(S)-HpETE intermediate primarily by 15-lipoxygenase 2 (15-LO-2) in macrophages, as well as the prostate, lung, and skin, but also by 15-LO-1 in airway epithelial cells, eosinophils, and reticulocytes.[1,2] It can also be produced by COX-1 in mast cells but is selectively metabolized by 15-hydroxy prostaglandin dehydrogenase (15-PGDH) into 15-oxoETE while the 15(R) isomer accumulates.[3,4] 15(S)-HETE is metabolized by 5-LO to form lipoxin A_4 (LXA_4) and LXB_4 *via* 5(S),15(S)-diH(p)ETE and 5(S),6(S)-epoxy-15(S)-HETE as intermediates.[5] It is involved in the initiation of angiogenesis by its involvement in the activation of various signaling pathways, including the Src and PI3K/AKT/mTOR pathways, as well as by HMG-CoA-mediated activation of the Rho GTPase Rac1.[6,7] Levels of 15(S)-HETE are reduced in human lung cancer tissue but are increased in the serum of patients with allergic asthma.[2,8]

About this Assay

Cayman's 15(S)-HETE ELISA Kit is a competitive assay that can be used for the quantification of 15(S)-HETE in plasma, serum, whole blood, and urine, as well as other sample matrices. The assay has a range of 78-10,000 pg/ml, with a midpoint (50% B/B_0) of 700-1,200 pg/ml, and a sensitivity (80% B/B_0) of approximately 185 pg/ml.

Principle Of This Assay

This assay is based on the competition between free 15(S)-HETE and a 15(S)-HETE-acetylcholinesterase (AChE) conjugate (15(S)-HETE-AChE Tracer) for a limited number of 15(S)-HETE polyclonal antibody binding sites. Because the concentration of the 15(S)-HETE-AChE Tracer is held constant while the concentration of free 15(S)-HETE varies, the amount of 15(S)-HETE-AChE Tracer that is able to bind to the 15(S)-HETE polyclonal antibody will be inversely proportional to the concentration of free 15(S)-HETE in the well. This antibody-15(S)-HETE complex binds to the mouse monoclonal 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 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of 15(S)-HETE-AChE Tracer bound to the well, which is inversely proportional to the amount of free 15(S)-HETE present in the well during the incubation as described in the equation:

$$\textbf{Absorbance} \propto \textbf{[Bound 15(S)-HETE-AChE Tracer]} \propto \textbf{1/[15(S)-HETE]}$$

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

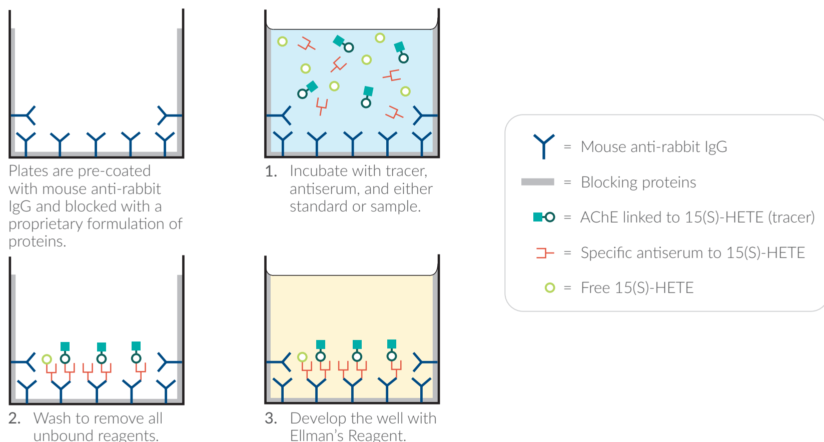


Figure 1. Schematic of the ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the 15(S)-HETE AChE-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B_0 (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

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

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

Dtn (Determination): one dtn is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

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

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

PRE-ASSAY PREPARATION

Buffer Preparation

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

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

   Dilute the contents of one vial of ELISA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

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

   5 ml vial Wash Buffer Concentrate (400X) (96-well kit; Item No. 400062): Dilute to a total volume of 2 L with ultrapure water and add 1 ml of Polysorbate 20 (Item No. 400035).

   OR

   12.5 ml vial Wash Buffer Concentrate (400X) (480-well kit; Item No. 400062): Dilute to a total volume of 5 L with ultrapure water and add 2.5 ml of Polysorbate 20 (Item No. 400035).

Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding 0.5 ml of Polysorbate 20 per 1 L of Wash Buffer (1X).

NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Preparation

Testing for Interference

This assay has been validated in 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 samples to obtain at least two different dilutions of each sample within the linear portion of the standard curve. If two different dilutions of the same sample show good correlation (differ by 20% or less) in the final calculated 15(S)-HETE concentration, sample purification is not required. If you do not see good correlation at the different dilutions, purification is advised. Purification methods will need to be determined by the end user and tested for compatibility in the assay. References for purification of arachidonic acid metabolites from biological samples are provided on page 31.[9,10]

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 be purified prior to use in this assay.

Plasma

Collect blood in vacutainers containing heparin, EDTA, or sodium citrate for plasma samples. To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes. Pipette off the top plasma layer without disturbing the white buffy layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Serum

Collect blood in vacutainers without a coagulant for serum samples. Allow samples to clot undisturbed for 30-60 minutes at RT. To obtain serum, centrifuge at 1-2,000 x g for 15-30 minutes. Pipette off the top serum layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Parallelism

To assess parallelism, human plasma, serum, and urine samples were serially diluted with ELISA Buffer (1X), and evaluated using the 15(S)-HETE ELISA Kit. Concentrations were plotted as a function of the sample dilution. The results are shown below.

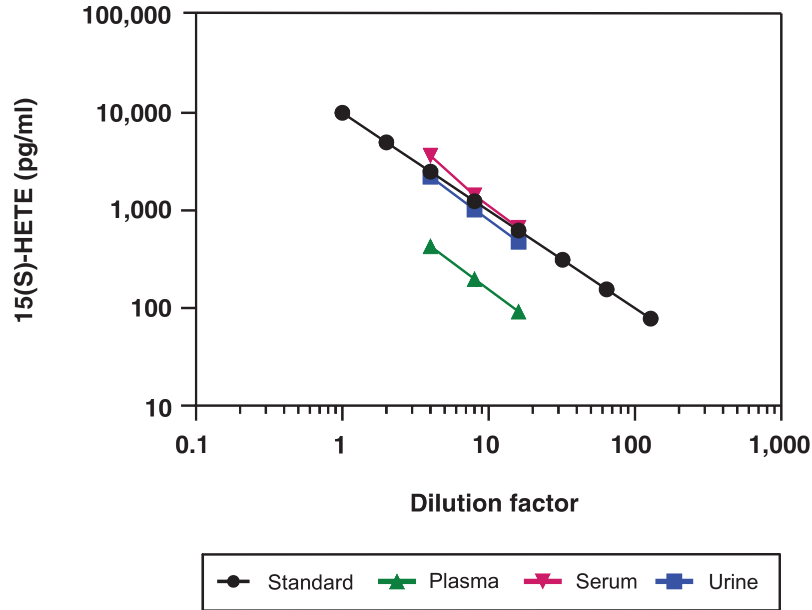


Figure 2. Parallelism of various matrices in the 15(S)-HETE ELISA

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

15(S)-HETE ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the 15(S)-HETE ELISA Standard (Item No. 434724) several times. Using the equilibrated pipette tip, transfer 100 µl of the standard into a clean test tube, then dilute with 900 µl ultrapure water. The concentration of this solution (the bulk standard) will be 50 ng/ml.

NOTE: If assaying culture media samples that have not been diluted with ELISA Buffer (1X), culture medium should be used in place of ELISA Buffer (1X) for dilution of the standard curve.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 800 µl ELISA Buffer (1X) to tube #1 and 500 µl ELISA Buffer (1X) to tubes #2-8. Transfer 200 µl of the bulk standard (50 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than 24 hours.

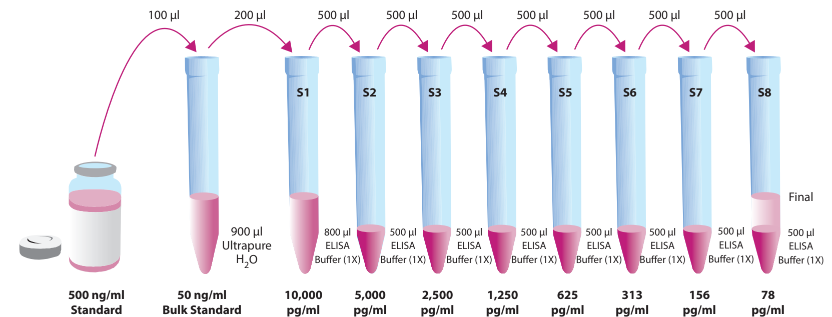


Figure 3. Preparation of the 15(S)-HETE standards

15(S)-HETE-AChE Tracer

Reconstitute the 15(S)-HETE-AChE Tracer (Item No. 434720) as follows:

100 dtn 15(S)-HETE-AChE Tracer (96-well kit): Reconstitute with 6 ml ELISA Buffer (1X).

OR

500 dtn 15(S)-HETE-AChE Tracer (480-well kit): Reconstitute with 30 ml ELISA Buffer (1X).

Store the reconstituted 15(S)-HETE-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). *NOTE: Do not store tracer with dye for more than 24 hours.*

15(S)-HETE ELISA Antiserum

Reconstitute the 15(S)-HETE ELISA Antiserum (Item No. 434722) as follows:

100 dtn 15(S)-HETE ELISA Antiserum (96-well kit): Reconstitute with 6 ml ELISA Buffer (1X).

OR

500 dtn 15(S)-HETE ELISA Antiserum (480-well kit): Reconstitute with 30 ml ELISA Buffer (1X).

Store the reconstituted 15(S)-HETE ELISA Antiserum at 4°C. It will be stable for at least four 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 or add 300 µl of dye to 30 ml of antiserum). *NOTE: Do not store antiserum 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 Blk, two NSB, and two B_0 wells, and an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at 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 4, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 23 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 4. Sample plate format

Performing the Assay

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

Addition of the Reagents

1. **ELISA Buffer**

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

2. **15(S)-HETE 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 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. **15(S)-HETE-AChE Tracer**

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

5. **15(S)-HETE ELISA Antiserum**

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

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate 18 hours at 4°C.

Development of the Plate

1. Reconstitute Ellman's Reagent (Item No. 400050) immediately before use (20 ml of reagent is sufficient to develop 100 wells):

   100 dtn vial Ellman's Reagent (96-well kit): Reconstitute with 20 ml of ultrapure water.

   OR

   250 dtn vial Ellman's Reagent (480-well kit): Reconstitute with 50 ml of ultrapure water.

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

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

ANALYSIS

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

Calculations

Preparation of the Data

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

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

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

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

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* 15(S)-HETE 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 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.

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

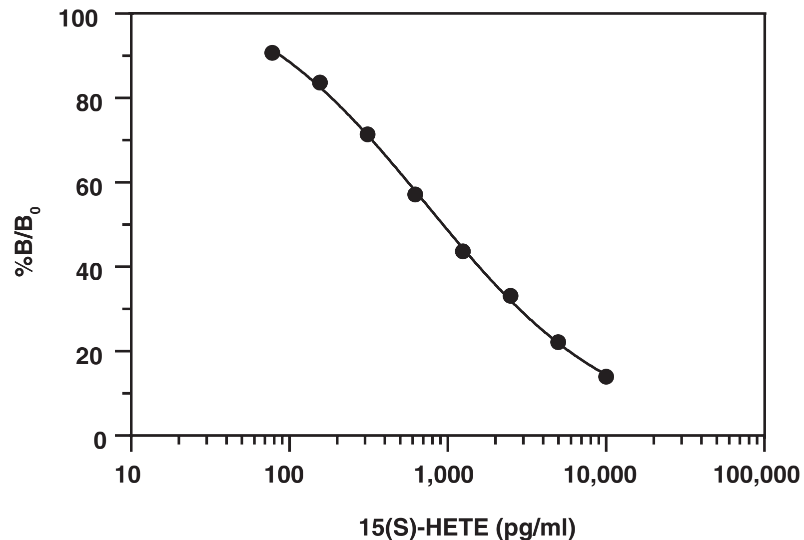
Representative Data

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 for 90 minutes

15(S)-HETE Standards (pg/ml)/ Controls	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	%B/B_0	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
NSB	0.012	--	--	--	--
B_0	0.937	0.925	--	--	--
TA	0.74	--	--	--	--
10,000.0	0.141	0.129	14.0	10.3	7.5
5,000.0	0.216	0.204	22.1	8.8	4.1
2,500.0	0.317	0.305	33.1	12.8	7.0
1,250.0	0.415	0.403	43.6	11.7	3.2
625.0	0.540	0.528	57.2	12.7	7.5
312.5	0.671	0.659	71.4	15.9	8.5
156.3	0.785	0.774	83.7	25.6**	7.8
78.1	0.852	0.840	90.7	32.5**	15.2

Table 1. 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 = 78-10,000 pg/ml
Sensitivity (defined as 80% B/B_0) = 185.4 pg/ml
Mid-point (defined as 50% B/B_0) = 935.8 pg/ml
Lower Limit of Detection (LLOD) = 58.9 pg/ml

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

Figure 5. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three human plasma controls in a single assay.

Matrix Control (pg/ml)	%CV
1,299.7	19.1
1,038.4	15.8
709.7	15.4

Table 2. Intra-assay precision

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

Matrix Control (pg/ml)	%CV
1,309.7	18.6
880.5	16.7
541.2	16.9

Table 3. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity	Compound	Cross Reactivity
15(S)-HETE	100%	5(R)-HETE	<0.01%
15(S)-HETrE	3.03%	5(S)-HETE	<0.01%
5(S),15(S)-DiHETE	2.87%	12(R)-HETE	<0.01%
15(S)-HEPE	0.93%	20-HETE	<0.01%
8(S),15(S)-DiHETE	0.35%	9(S)-HODE	<0.01%
(±)15-HEPE	0.21%	13(R)-HODE	<0.01%
Arachidonic Acid	0.17%	Leukotriene B_4	<0.01%
15(R)-HETE	0.08%	Prostaglandin D_2	<0.01%
12(S)-HETE	0.04%	Prostaglandin E_2	<0.01%
14,15-DiHETrE	0.03%	6-keto Prostaglandin $F_{1\alpha}$	<0.01%
13(S)-HODE	0.02%	Prostaglandin $F_{2\alpha}$	<0.01%
(±)14,15-EpETrE	<0.01%	Thromboxane B_2	<0.01%

Table 4. Cross reactivity of the 15(S)-HETE ELISA

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (>10% of B_0)	A. Poor washing B. Exposure of NSB wells to specific antibody
Very low B_0	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Low sensitivity (shift in dose-response curve)	A. Standard is degraded or contaminated B. Dilution error in preparing standards
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present
Low signal in the sample wells (below the range of the standard curve)	A. AChE inhibitors are present; ensure that the samples and buffers are free of AChE inhibitors B. Sample requires further dilution
Only TA wells develop	A. Trace organic contaminants in the water source B. The tracer was not added to the wells

References

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15(S)-HETE Assay Summary					
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
15(S)-HETE-AChE Tracer	--	--	50 µl	50 µl	50 µl
15(S)-HETE Antiserum	--	--	--	50 µl	50 µl
Incubate	Seal the plate and incubate for 18 hours at 4°C				
Aspirate	Aspirate wells and wash 5 x ~300 µl with 15(S)-HETE Wash Buffer (1X)				
Apply Ellman's Reagent	200 µl	200 µl	200 µl	200 µl	200 µl
TA - Apply Tracer	--	5 µl	--	--	--
Develop	Seal the plate and incubate for 90-120 minutes at RT on orbital shaker protected from light				
Read	Read absorbance at 405-420 nm				

Table 5. Assay summary

NOTES

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