12(S)-HETE ELISA Kit

Item No. 534571

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
534572	12(S)-HETE ELISA Polyclonal Antiserum	1 vial/100 dtn	-20°C
534573	12(S)-HETE-AChE Tracer	1 vial/100 dtn	-20°C
534574	12(S)-HETE ELISA Standard	1 vial	-20°C
400060	ELISA Buffer Concentrate (10X)	1 vial/10 ml	RT
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400004/400006	Precoated (Mouse Anti-Rabbit IgG) ELISA 96-Well Strip/Solid Plate	1 plate	4°C
400012	96-Well Cover Sheet	1 ea	RT
400050	Ellman's Reagent	3 vials/100 dtn	-20°C
400040	ELISA Tracer Dye	1 ea	RT
400042	ELISA Antiserum Dye	1 ea	RT

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 12(S)-HETE ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed 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 between 405-420 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. An orbital microplate shaker
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: Ultrapure water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 12)

INTRODUCTION

Background

12(S)-HETE is a hydroxy fatty acid formed *via* oxidation of arachidonic acid to 12(S)-HpETE by 12-lipoxygenase (12-LO) followed by reduction of the hydroxyl moiety in platelets and endothelial cells, but also by leukocyte 12/15-LO in murine macrophages in response to IL-4 or IL-13 stimulation.[1-4] It signals through various receptors and signaling pathways, including the 12(S)-HETE receptor (12-HETER), leukotriene B_4 receptor 2 (BLT2), VE-cadherin, NF-κB, Rho-associated kinase (ROCK), and c-Jun N-terminal kinase 1 (JNK1), to modify inflammatory processes, itch perception, vascular contraction, insulin signaling, and cell migration.[2,3,5] 12(S)-HETE is produced in platelets in response to various agonists, including ADP, thromboxane A_2 (TXA_2), and collagen.[6] Plasma and intraplatelet levels of 12(S)-HETE are correlated with platelet aggregation and pharmacological inhibition of 12-LOX reduces platelet recruitment, thrombus formation, and vessel occlusion in mouse models of arteriole thrombosis but has no effect on laser ablation-induced hemostatic clot formation in mice.[7] Platelet production and urinary excretion of 12(S)-HETE are increased in patients with essential hypertension.[8] 12(S)-HETE induces cancer cell migration in numerous in vitro and mouse models and levels of 12(S)-HETE are positively correlated with colon cancer malignancy.[5]

About this Assay

Cayman's 12(S)-HETE ELISA Kit is a competitive assay that can be used for the quantification of 12(S)-HETE in plasma and serum. The assay has a range of 0.091-200 ng/ml, with a midpoint (50% B/B_0) of 3.0-6.5 ng/ml, and a sensitivity (80% B/B_0) of approximately 0.5 ng/ml.

Principle Of This Assay

This assay is based on the competition between free 12(S)-HETE and a 12(S)-HETE-acetylcholinesterase (AChE) conjugate (12(S)-HETE-AChE Tracer) for a limited number of 12(S)-HETE polyclonal antibody binding sites. Because the concentration of the 12(S)-HETE-AChE Tracer is held constant while the concentration of free 12(S)-HETE varies, the amount of 12(S)-HETE-AChE Tracer that is able to bind to the 12(S)-HETE polyclonal antibody will be inversely proportional to the concentration of free 12(S)-HETE in the well. This antibody-12(S)-HETE 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 12(S)-HETE-AChE Tracer bound to the well, which is inversely proportional to the amount of free 12(S)-HETE present in the well during the incubation, as described in the equation:

$$\text{Absorbance} \propto \text{[bound 12(S)-HETE-AChE tracer]} \propto 1/\text{[12(S)-HETE]}$$

A schematic of this process is shown in Figure 1 on page 9.

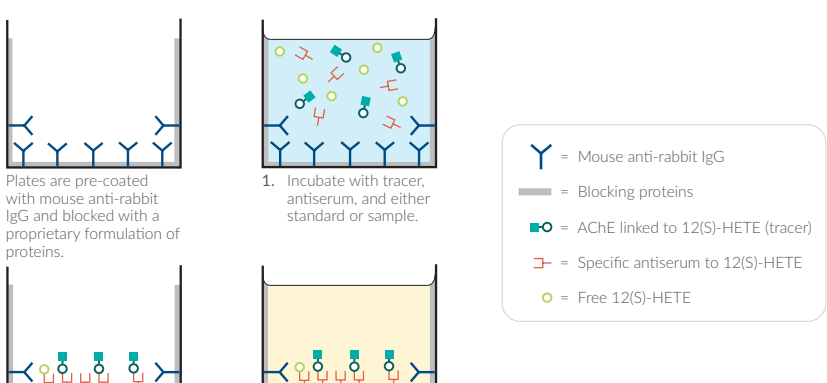


Figure 1. Schematic of the ELISA

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 12(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:

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

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**

   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. 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

This assay has been validated in human plasma and serum without purification. It is recommended that plasma and serum samples be diluted at least 1:5 into ELISA Buffer (1X) prior to testing in the assay. Purification may be needed if the 12(S)-HETE levels are very low and an absolute quantification is required. Please see the optional plasma/serum purification protocol on page 14. EDTA plasma is recommended. Other matrices may cause interference and require sample purification or dilution determined by the end user. Please read this section thoroughly before beginning the assay.

Plasma

Collect blood in vacutainers containing EDTA. 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. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1-2,000 x g for 15-30 minutes. Pipette off the serum layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Testing for Interference

This assay has been validated in human plasma and serum. 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 12(S)-HETE concentration, sample 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. See page 14 for one such method.

Sample Purification Protocol (optional)

1. Aliquot a known amount of each sample into a clean test tube (500 µl is recommended).
2. Add 4X the sample volume of ice-cold acetone and vortex thoroughly. Incubate on ice for 5 minutes and centrifuge at 10,000 x g for 10 minutes. Transfer the supernatant to a clean test tube.
3. Evaporate acetone under a gentle stream of nitrogen without heat.
4. Resuspend the extract in ELISA Buffer (1X) to its original volume, and use this for ELISA analysis. Purified plasma and serum may need to be diluted at least 1:2 with ELISA Buffer (1X) to fall within the linear range of 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 be purified prior to use in this assay.

Parallelism

To assess parallelism, plasma and serum were serially diluted with ELISA Buffer (1X) and evaluated using the 12(S)-HETE ELISA Kit. Measured concentrations were plotted as a function of the sample dilution. The results are shown below.

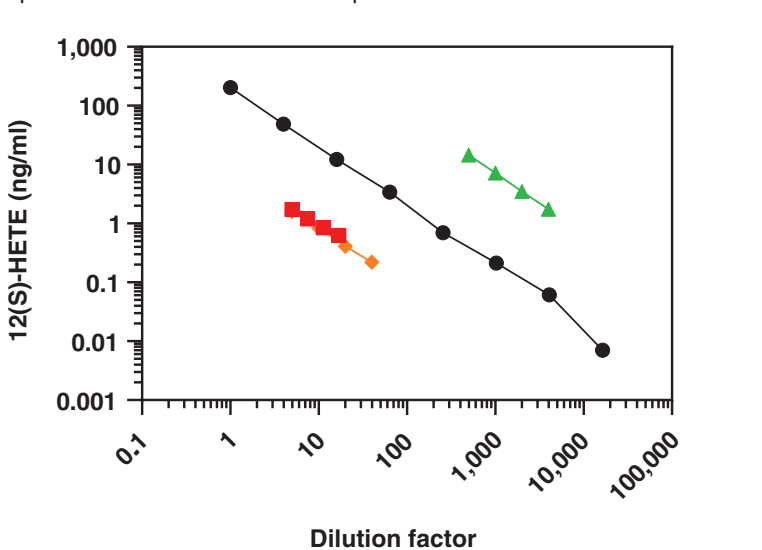


Figure 2. Parallelism of human plasma and serum in the 12(S)-HETE ELISA

Spike and Recovery

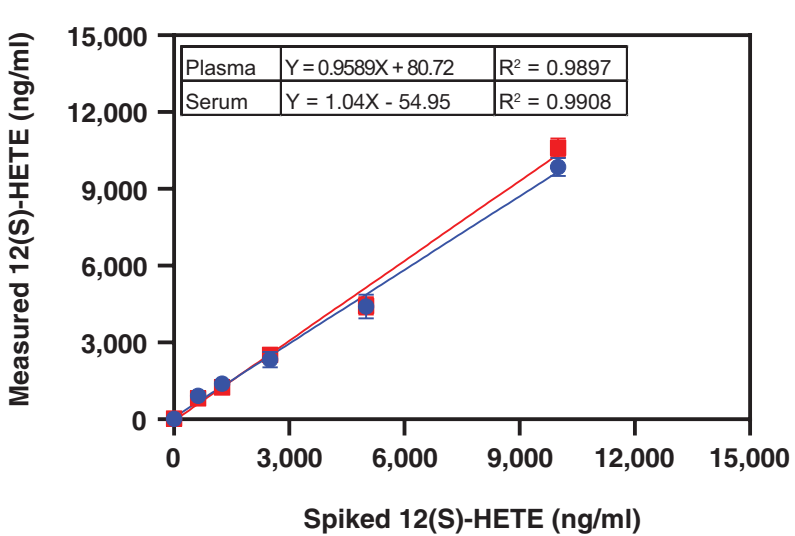


Figure 3. Spike and recovery of 12(S)-HETE (high) in human plasma and serum
Plasma and serum were spiked with different amounts of 12(S)-HETE, serially diluted with ELISA Buffer (1X), and evaluated using the 12(S)-HETE ELISA. The error bars represent standard deviations obtained from multiple dilutions of each sample.

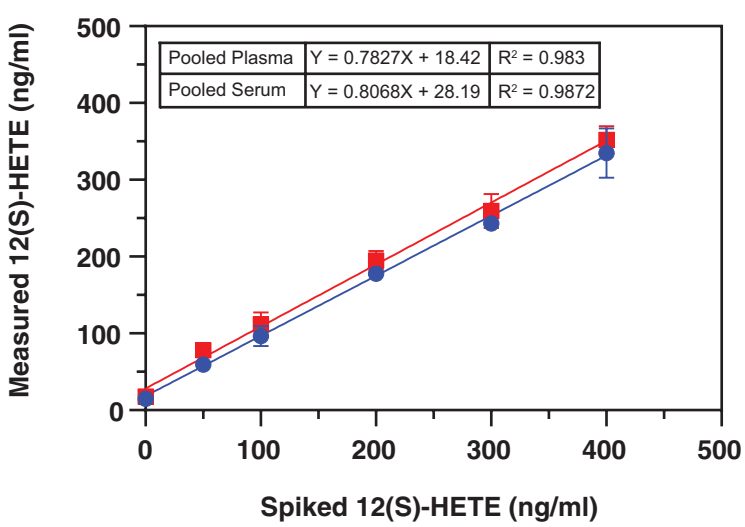


Figure 4. Spike and recovery of 12(S)-HETE (low) in human plasma and serum
Plasma and serum samples were spiked with different amounts of 12(S)-HETE, purified as described on page 14, then serially diluted with ELISA Buffer (1X) and evaluated using the 12(S)-HETE ELISA.

Linearity

Plasma and serum samples were spiked with 10 µg/ml 12(S)-HETE, serially diluted with ELISA Buffer (1X), and evaluated for linearity using the 12(S)-HETE ELISA Kit. The results are shown in Table 1 below.

Dilution Factor	Measured Concentration (µg/ml)	Linearity (%)
Plasma		
2,000	9.8	100
4,000	9.7	99
8,000	9.5	97
Serum		
2,000	10.1	100
4,000	11.0	109
8,000	10.6	105
16,000	10.6	105

Table 1. Linearity in human plasma and serum

NOTE: Linearity has been calculated using the following formula:
*%Linearity = (Observed concentration value, dilution adjusted/First observed concentration value in the dilution series, dilution adjusted)*100*

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

12(S)-HETE ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with the 12(S)-HETE ELISA Standard (Item No. 534574) several times. Using the equilibrated pipette tip, transfer 100 µl of the standard into a clean test tube, then dilute with 900 µl of ELISA Buffer (1X). The concentration of this solution (the bulk standard) will be 2,000 ng/ml. This bulk standard should not be stored for more than 1 hour.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 900 µl of ELISA Buffer (1X) to tube #1 and 400 µl of ELISA Buffer (1X) to tubes #2-8. Transfer 100 µl of the bulk standard (2,000 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 200 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 200 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should be used within 1 hour.

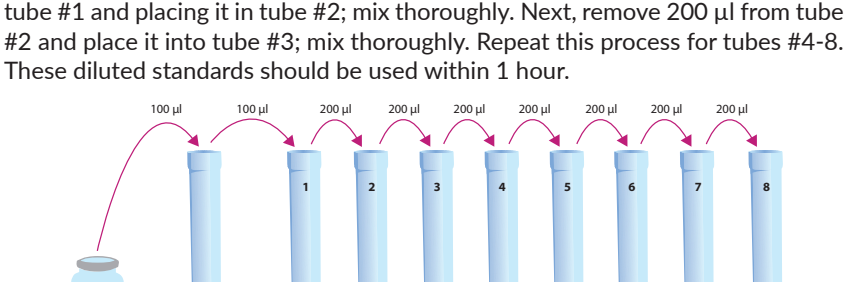


Figure 5. Preparation of the 12(S)-HETE standards

12(S)-HETE-AChE Tracer

Reconstitute the 12(S)-HETE-AChE Tracer (Item No. 534573) with 6 ml of ELISA Buffer (1X). Store the reconstituted 12(S)-HETE-AChE Tracer at 4°C (***do not freeze!***). It will be stable for at least 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). *NOTE: Do not store tracer with dye for more than 3 weeks at 4°C.*

12(S)-HETE ELISA Polyclonal Antiserum

Reconstitute the 12(S)-HETE Polyclonal Antiserum (Item No. 534572) with 6 ml of ELISA Buffer (1X). Store the reconstituted 12(S)-HETE Polyclonal Antiserum at 4°C (***do not freeze!***). 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). *NOTE: Do not store antiserum with dye for more than 3 weeks 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 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 24 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 wells
TA - Total Activity well
NSB - Non-Specific Binding wells
B_0 - Maximum Binding wells
S1-S8 - Standard 1-8 wells
1-24 - Sample wells

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. **ELISA Buffer**

   Add 150 µl of ELISA Buffer (1X) to NSB wells. Add 100 µl of ELISA Buffer (1X) to B_0 wells.

2. **12(S)-HETE ELISA Standard**

   Add 100 µl from tube #8 to both of the lowest standard wells (S8). Add 100 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 100 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. **12(S)-HETE-AChE Tracer**

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

5. **12(S)-HETE 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 Cover Sheet (Item No. 400012) and incubate 2 hours at room temperature on an orbital shaker.

Development of the Plate

1. Reconstitute Ellman's Reagent (Item No. 400050) immediately before use. Reconstitute 100 dtn vial with 20 ml of ultrapure water.

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

2. Empty the wells and rinse five times with ~300 µl of Wash Buffer (1X).
3. Add 200 µl of Ellman's Reagent to each well.
4. Dilute the reconstituted tracer 1:2 by mixing 50 µl of tracer with 50 µl ELISA buffer (1X), and add 5 µl of it 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 ≥0.6 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 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 0.5 A.U. (blank subtracted). The plate should be read when the absorbance of the B_0 wells in the range of 0.5-1.5 A.U. (blank subtracted). If the absorbance of the wells exceeds 2.0 A.U., wash the plate, add fresh Ellman's Reagent and let it develop again.

ANALYSIS

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

Calculations

Preparation of the Data

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

NOTE: If the plate reader has not subtracted the absorbance readings of the 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* 12(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.

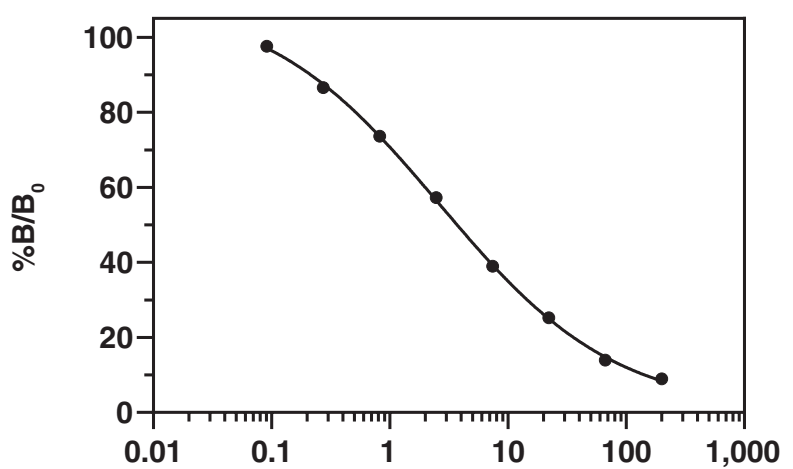
Absorbance at 414 nm

12(S)-HETE Standards (ng/ml)/ Controls	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	%B/B_0	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
NSB	0.012	--	--	--	--
B_0	0.990	0.978	--	--	--
200	0.099	0.087	8.9	6.6	15.6
66.7	0.148	0.136	14.0	8.2	15.1
22.2	0.258	0.246	25.2	6.9	9.5
7.41	0.393	0.381	39.0	8.4	6.7
2.5	0.572	0.560	57.3	9.2	5.6
0.823	0.732	0.720	73.6	14.8	4.9
0.274	0.859	0.847	86.6	19.4	13.6
0.091	0.967	0.955	97.6	32.1**	20.7**
TA	2.15	--	--	--	--

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 = 0.091-200 ng/ml
Sensitivity (defined as 80% B/B_0) = 0.52 ng/ml
Mid-point (defined as 50% B/B_0) = 3.7 ng/ml
LLOD = 0.32 ng/ml

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

Figure 7. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of two human serum (high, medium) and one human EDTA plasma (low) controls in a single assay.

Matrix Control (ng/ml)	%CV
4,092.8	13.7
85.9	10.2
33.6	9.6

Table 3. Intra-assay precision

Inter-assay precision was determined by analyzing two human serum (high, medium) and one human EDTA plasma (low) controls in eight separate assays on three different days.

Matrix Control (ng/ml)	%CV
2,961	11.3
77.3	5.5
30.9	4.5

Table 4. Inter-assay precision

Cross Reactivity:

Compound	Cross-Reactivity
12(S)-HETE	100%
12(S)-HETrE	64%
5(S),12(S)-DiHETE***	23.3%
12(S)-HpETE	5.1%
Leukotriene B_4	3.8%
15(S)-HETE	2.5%
12-OxoETE	1.1%
12(R)-HETE	0.11%
5(R)-HETE	0.01%
12(S)-HHTrE	<0.01%
5(S)-HETE	<0.01%
Arachidonic Acid	<0.01%
Lipoxin B_4	<0.01%
Thromboxane B_2	<0.01%
Prostaglandin D_2	<0.01%
Prostaglandin E_2	<0.01%
Prostaglandin $F_{2\alpha}$	<0.01%

Table 5. Cross reactivity of the 12(S)-HETE ELISA
****Not reported in human plasma or serum*

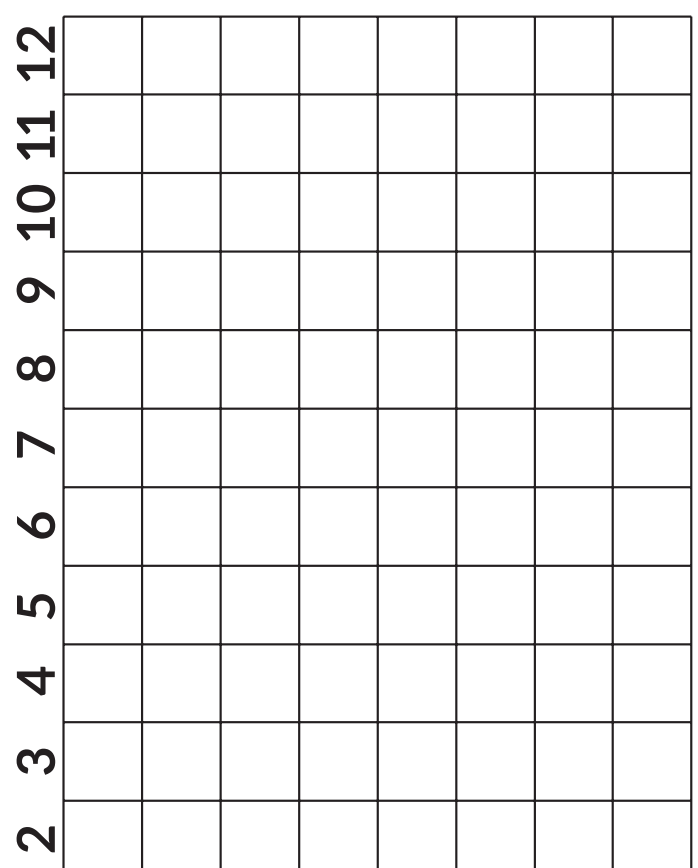
RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (>10% of B_0)	A. Poor washing B. Exposure of NSB wells to specific antiserum
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

12(S)-HETE Assay Summary					
Procedure	**Blk**	**TA**	**NSB**	**B_0**	**Standards/ Samples**
Reconstitute and mix	Mix all reagents gently				
ELISA Buffer (1X)	--	--	150 µl	100 µl	--
Standards/Samples	--	--	--	--	100 µl
12(S)-HETE-AChE Tracer	--	--	50 µl	50 µl	50 µl
12(S)-HETE Polyclonal Antiserum	--	--	--	50 µl	50 µl
Incubate	Seal the plate and incubate for 2 hours at room temperature				
Aspirate	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 Diluted Tracer	--	5 µl	--	--	--
Develop	Seal the plate and incubate for 60 minutes at room temperature on orbital shaker protected from light				
Read	Read absorbance at 414 nm				

Table 5. Assay summary



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NOTES

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