(±)12(13)-DiHOME ELISA Kit

Item No. 501720

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Customer Service 800.364.9897
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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size
401722	(±)12(13)-DiHOME ELISA Antiserum	1 vial/100 dtn
401720	(±)12(13)-DiHOME AChE Tracer	1 vial/100 dtn
401724	(±)12(13)-DiHOME ELISA Standard	1 vial
400060	ELISA Buffer Concentrate (10X)	1 vial/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
400035	Polysorbate 20	1 vial/3 ml
400004/400006	Mouse Anti-Rabbit IgG Coated Plate	1 plate
400050	Ellman's Reagent	3 vials/100 dtn
400040	ELISA Tracer Dye	1 each
400042	ELISA Antiserum Dye	1 each
400012	96-Well Cover Sheet	1 cover

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

Fax: 734-971-3640

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 at 414 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass-distilled water or deionized water is acceptable *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000)*
4. Materials used for **Sample Preparation** (see page 13)

INTRODUCTION

Background

(±)12(13)-DiHOME is the diol form of (±)12(13)-EpOME, a cytochrome P450-derived epoxide of linoleic acid also known as isoleukotoxin.[1] It is formed from 12(13)-EpOME by soluble epoxide hydrolase (sEH) in neutrophils.[2] 12(13)-DiHOME levels are elevated in bronchoalveolar lavage fluid (BALF) in humans following exposure to biodiesel exhaust and in exhaled breath condensate in patients with asthma following allergen exposure.[3,4] Plasma levels of 12(13)-DiHOME are increased immediately following moderate-intensity exercise in mice and humans, an effect that can be prevented by brown adipose tissue removal in the mouse.[5] It is also increased in rat spinal cord following burn injury, and it enhances cold tolerance, increases fatty acid uptake into brown adipocytes, and decreases serum triglyceride levels in mice.[6,7] 12(13)-DiHOME is toxic to *Sf*21 cells expressing sEH and to lacZ-expressing control cells, unlike isoleukotoxin, which is only toxic to cells containing sEH.[1,2] A mixture of 12(13)- and 9(10)-DiHOME induces cell death in rabbit renal proximal tubule cells concomitant with mitochondrial respiration dysfunction and induces lung injury, respiratory distress, and mortality in mice.[8,9] However, alone, 12(13)-DiHOME has no effect on mitochondrial respiration or the mitochondrial membrane potential in rabbit renal cortical mitochondria and increases mitochondrial respiration, as well as fatty acid uptake and oxidation, in C2C12 myotubes.[5,10]

About This Assay

Cayman's (±)12(13)-DiHOME ELISA Kit is a competitive assay that can be used for quantification of 12(13)-DiHOME in plasma, serum, and BALF. The assay has a range of 0.05-100 ng/ml and a sensitivity (80% B/B_0) of approximately 0.3 ng/ml.

Principle of the Assay

This assay is based on the competition between native 12(13)-DiHOME and a (±)12(13)-DiHOME acetylcholinesterase (AChE) conjugate ((±)12(13)-DiHOME AChE Tracer) for a limited amount of (±)12(13)-DiHOME ELISA Antiserum. Because the concentration of the (±)12(13)-DiHOME AChE Tracer is held constant while the concentration of native 12(13)-DiHOME varies, the amount of (±)12(13)-DiHOME AChE Tracer that binds to the (±)12(13)-DiHOME ELISA Antiserum will be inversely proportional to the concentration of native 12(13)-DiHOME in the well. This antibody 12(13)-DiHOME 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(13)-DiHOME AChE Tracer bound to the well, which is inversely proportional to the amount of free 12(13)-DiHOME present in the well during the incubation as described in the equation:

$$\text{Absorbance} \propto \text{[Bound (±)12(13)-DiHOME AChE Tracer]} \propto 1/\text{[12(13)-DiHOME]}$$

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

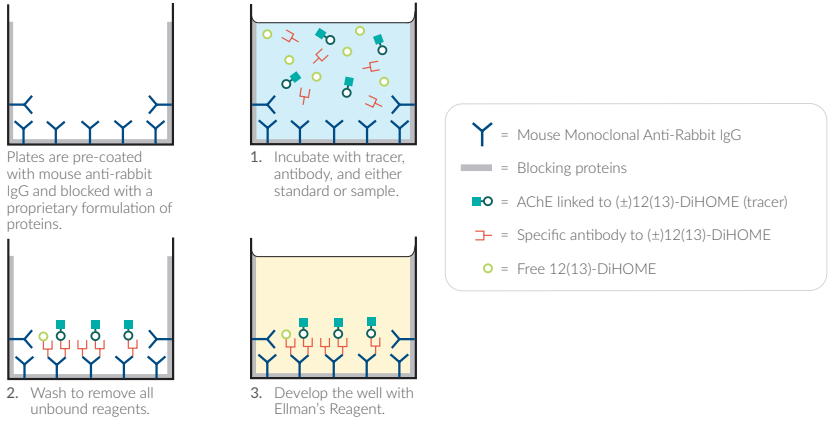


Figure 1. Schematic of the (±)12(13)-DiHOME ELISA

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 NSB wells.

TA (Total Activity): total enzymatic activity of the 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. Do not forget to subtract the Blk absorbance values.

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 particular sample or standard well to that of the maximum binding (B_0) well.

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 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 point two standard deviations away from 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 (10X) (Item No. 400060) with 90 ml of pure 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 pure water.*

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

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with pure 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: 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 tested using human plasma, serum, and BALF. Other samples types should be checked 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 11 ng/ml and 0.3 ng/ml (*i.e.*, between 20-80% B/B_0, which is the linear portion of the standard curve). The two different dilutions of the sample should show good correlation (differ by 20% or less) in the final calculated 12(13)-DiHOME concentration.

Plasma, Serum, and BALF

Plasma, serum, and BALF samples may require extraction prior to quantification in the assay. Sample extraction should be performed using a method similar to the following protocol.

1. To a 500 µl sample, add 125 µl of 2M HCl and 2 ml of pure water. Incubate at room temperature for 15 minutes.
2. Equilibrate a 6 ml C18 column (Item No. 400020) with 3 ml of 100% ethanol (EtOH) followed by 3 ml of pure water.
3. Apply the sample to the column.
4. Wash with 6 ml pure water followed by 6 ml of 15% ethanol in pure water. Discard washes.
5. Elute the sample slowly by applying 3 ml ethyl acetate. Collect the eluant in a polypropylene tube.
6. Evaporate to dryness using a centrifugal concentrator under vacuum.
7. Reconstitute with 500 µl of the assay buffer and quantify immediately. *NOTE: If samples cannot be quantified immediately, store at -20°C.*

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.

Sample Matrix Properties

Linearity

Human plasma, serum, and BALF samples were serially diluted and evaluated for linearity using the (±)12(13)-DiHOME ELISA Kit. The results are shown in the table on page 15.

Non-extracted Sample			Sample Extracted Over C18 Column		
Dilution	Concentration (ng/ml)	Dilutional Linearity (%)	Dilution	Concentration (ng/ml)	Dilutional Linearity (%)
Plasma			Plasma		
1:27	174	129	1:3	8.4	118
1:81	125	93	1:9	5.7	80
1:243	134	100	1:27	7.1	100
Serum			Serum		
1:27	208	141	1:3	9.1	123
1:81	161	109	1:9	6.1	83
1:243	148	100	1:27	7.1	100
BALF					
1:2	29	96			
1:4	25	84			
1:8	31	102			
1:16	30	100			

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

Spike and Recovery

Human plasma and serum samples were spiked with (±)12(13)-DiHOME, extracted, and diluted as described in the Sample Preparation section and analyzed using the (±)12(13)-DiHOME ELISA Kit. Additionally, plasma, serum, and BALF samples were spiked with (±)12(13)-DiHOME, diluted without extraction, and analyzed using the (±)12(13)-DiHOME ELISA Kit. The results are shown below. The y-intercept corresponds to the amount of endogenous 12(13)-DiHOME in the sample.

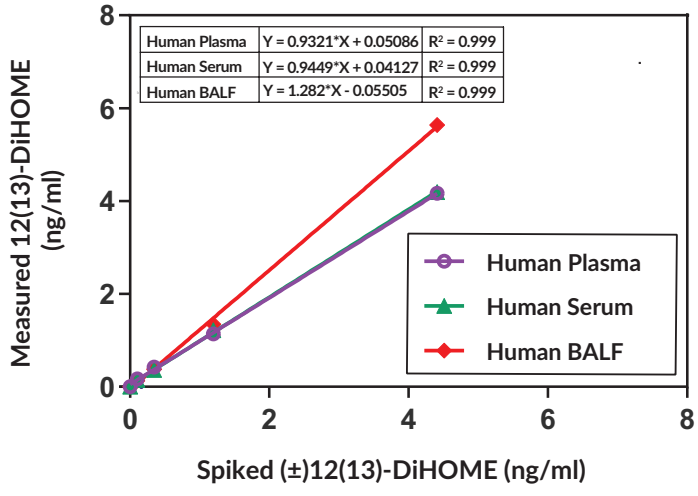


Figure 2. Spike and recovery of non-extracted human plasma, serum, and BALF

	Non-extracted Sample		Sample Extracted Over C18 Column	
Sample	Spike Concentration (ng/ml)	% Recovery	Spike Concentration (ng/ml)	% Recovery
Plasma	4.5	84	200	97
	1.5	100	50	89
	0.5	108	10	79
	0.15	122	1	104
Serum	4.5	85	200	90
	1.5	107	50	79
	0.5	92	10	103
	0.15	105	1	118
BALF	4.5	114		
	1.5	118		
	0.5	96		
	0.15	91		

Table 2. Spike and recovery of non-extracted human plasma, serum, and BALF

Parallelism

To assess parallelism, human plasma, serum, and BALF samples were extracted and serially diluted, or serially diluted without extraction, and evaluated using the (±)12(13)-DiHOME ELISA Kit. Concentrations were plotted as a function of the sample dilution. The results are shown below. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted human plasma, serum, and BALF samples. Non-extracted plasma and serum samples show a linear and parallel response to the standard curve, however, the concentrations are higher than what is commonly reported in the literature. Once extracted, these values fall within the more commonly reported ranges for 12(13)-DiHOME endogenous concentrations.

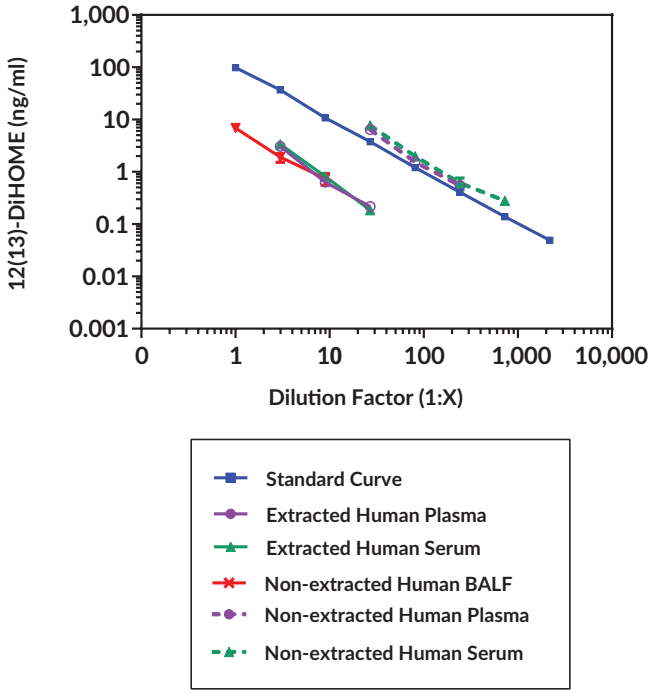


Figure 3. Parallelism of sample matrices in the (±)12(13)-DiHOME ELISA Kit

Preparation of Assay-Specific Reagents

(±)12(13)-DiHOME ELISA Standard

Equilibrate a pipette tip by repeatedly filling and expelling the tip with (±)12(13)-DiHOME ELISA Standard (Item No. 401724) several times. Using the equilibrated pipette tip, transfer 100 µl of the standard into a clean test tube, then dilute with 900 µl ELISA Buffer (1X). The concentration of this solution (the bulk standard) will be 1 µg/ml. This bulk standard should not be stored for more than two hours.

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 (1 µg/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 300 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 300 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.

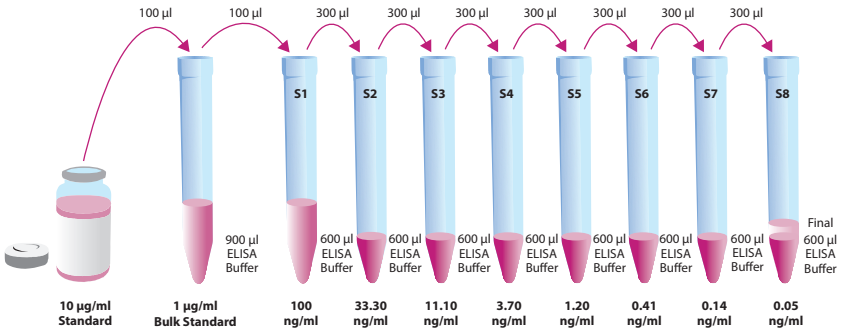


Figure 4. Preparation of the (±)12(13)-DiHOME Standards

(±)12(13)-DiHOME AChE Tracer

Reconstitute the (±)12(13)-DiHOME AChE Tracer (Item No. 401720) with 6 ml of ELISA Buffer (1X). Store the reconstituted (±)12(13)-DiHOME 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). *NOTE: Do not store tracer with dye for more than 24 hours.*

(±)12(13)-DiHOME ELISA Antiserum

Reconstitute the (±)12(13)-DiHOME ELISA Antiserum (Item No. 401722) with 6 ml of ELISA Buffer (1X). Store the reconstituted (±)12(13)-DiHOME ELISA Antiserum at 4°C (*do not freeze!*) and use within 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 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 of 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 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 36 for more details). We suggest you record the contents of each well on the template sheet provided (see page 37).

	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. **(±)12(13)-DiHOME 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. **(±)12(13)-DiHOME AChE Tracer**

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

5. **(±)12(13)-DiHOME ELISA 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 two 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 pure water.
2. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X).
3. Add 200 µl of Ellman's Reagent Solution to each well.
4. Add 5 µl of the reconstituted tracer to the TA wells.
5. Cover the plate with 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 for 90 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 of 414 nm.

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 Chemical has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysisTools/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.)

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* (±)12(13)-DiHOME 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 concentration of the sample prior to the 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.

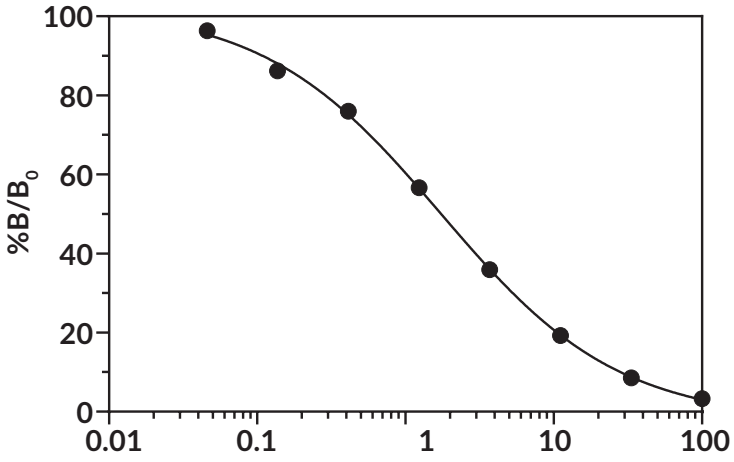
NOTE: If there is an error in the B_0 wells, it is possible to calculate sample concentrations by plotting the absorbance values and back calculating sample absorbance off the standard curve.

Performance Characteristics

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 (90 minutes)**

(±)12(13)-DiHOME Standards (ng/ml)	Blank-subtracted Absorbance	NSB-corrected Absorbance	%B/B_0	%CV Intra-assay Precision*	%CV Inter-assay Precision*
NSB	0.008	--	--	--	--
B_0	0.995	0.987	--	--	--
100.00	0.041	0.033	3.3	10.5	11.2
33.30	0.093	0.085	8.5	8.0	5.7
11.10	0.198	0.190	19.2	5.7	3.6
3.70	0.363	0.355	35.9	6.7	1.6
1.23	0.567	0.559	56.6	8.3	2.7
0.41	0.759	0.751	76.0	9.8	6.7
0.14	0.859	0.851	86.2	13.1†	12.2†
0.05	0.959	0.951	96.3	28.5†	15.2†
TA	1.154	--	--	--	--

Table 3. 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 = 0.05-100 ng/ml
Sensitivity (defined as 80% B/B_0) = 0.289 ng/ml
Mid-point (defined as 50% B/B_0) = 1.749 ng/ml
Lower Limit of Detection (LLOD) = 0.043 ng/ml

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

Figure 6. Typical standard curve

Precision:

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

Matrix Control (ng/ml)	%CV Intra-assay variation
7.84	8.6
2.03	6.5
0.58	8.9

Table 4. Intra-assay Precision

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

Matrix Control (ng/ml)	%CV Inter-assay variation
7.87	12.5
1.62	12.5
0.47	8.1

Table 5. Inter-assay Precision

Cross Reactivity:

Compound	Cross Reactivity
(±)12(13)-DiHOME	100%
(±)9(10)-DiHODE	0.30%
(±)12(13)-EpOME	0.16%
9-OxoODE	0.03%
(±)13-HpODE	0.03%
5(S)-HETE	0.02%
(±)11(12)-EET	0.02%
(±)14(15)-EET	0.02%
(±)12-HETE	<0.03%
(±)9-HODE	<0.03%

Table 6. Cross reactivity of the (±)12(13)-DiHOME ELISA Kit

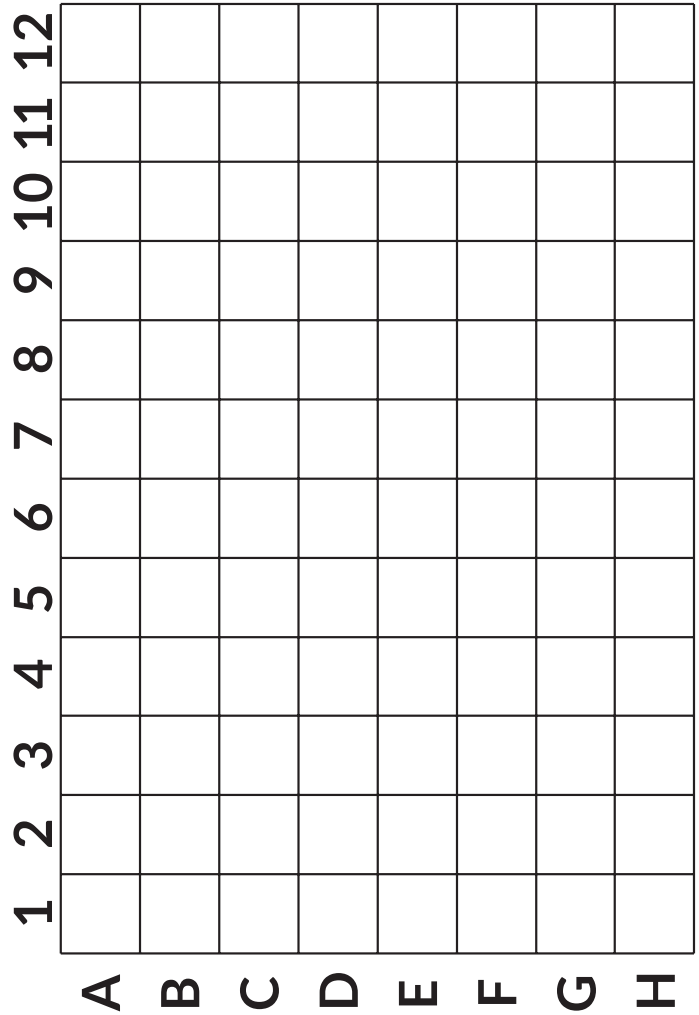
RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (>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)	Standard is degraded or contaminated
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present - consider sample purification prior to analysis

(±)12(13)-DiHOME 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
(±)12(13)-DiHOME AChE Tracer	-	-	50 µl	50 µl	50 µl
(±)12(13)-DiHOME ELISA Antiserum	-	-	-	50 µl	50 µl
Seal	Seal the plate				
Incubate	Incubate 2 hours at room temperature on an orbital shaker				
Aspirate	Aspirate wells and wash 5 x ~300 µl				
Ellman's Reagent	200 µl	200 µl	200 µl	200 µl	200 µl
(±)12(13)-DiHOME AChE Tracer	-	5 µl	-	-	-
Seal	Seal plate and incubate for 90 minutes at room temperature on an orbital shaker, protect from light				
Read	Remove plastic seal and read absorbance at 414 nm				

Table 7. Assay Summary



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NOTES

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