Tribioscience

(±)12(13)-DiHOME ELISA (Catalog Number: TBS32129)

ELISA for determination of (±)12(13)-DiHOME concentrations in biological samples.

Introduction

(±)12(13)-DiHOME is the vicinal diol metabolite of (±)12(13)-EpOME (i.e., isoleukotoxin), an epoxide derived from linoleic acid via cytochrome P450 epoxygenase activity. It has been shown to suppress neutrophil respiratory burst activity and exhibit selective cytotoxicity in certain cell lines expressing sEH. Thus, (±)12(13)-DiHOME bridges lipid metabolism and immune modulation, acting both as a downstream stress-responsive signal and as a paracrine regulator in inflammation and energy homeostasis.

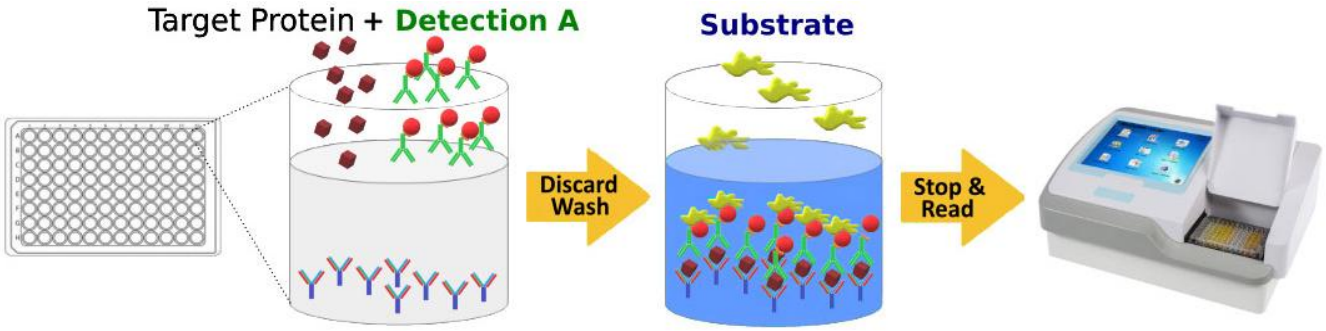
Intended Use

The Tribioscience's (±)12(13)-DiHOME ELISA Kit utilizes competitive ELISA technologies for the quantitative analysis of (±)12(13)-DiHOME in biological samples. It provides a vital tool to accurately measure (±)12(13)-DiHOME with applications in inflammation, metabolism, and respiratory research. The assay has a range of 0.14-100 ng/ml.

Assay Principle

Tribioscience's (±)12(13)-DiHOME ELISA Kit is a competitive enzyme-labeled immunoassay (Fig. 1). The 96-well microtiter plate is pre-coated with an anti-(±)12(13)-DiHOME antibody. During the assay, (±)12(13)-DiHOME standard solution or samples are added to each well, followed by adding horse radish peroxidase (HRP) -(±)12(13)-DiHOME conjugate, which will compete with (±)12(13)-DiHOME in standard or sample for binding to antibody during the incubation. After plate wash, an ultra-sensitive HRP substrate is added to the wells leading to a colored product only in the presence of HRP, and optical density is inversely related to (±)12(13)-DiHOME concentrations in the samples. The accurate concentration of (±)12(13)-DiHOME can then be determined by interpolation using the standard curve constructed in the same run.

Fig. 1. Simple Procedures



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Pre-coated Microplate	TBS32129A	96 well microplate (12 strips of 8 wells) coated with an antibody specific for (±)12(13)-DiHOME.	Return unused wells to the foil pouch. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
(±)12(13)-DiHOME Standard	TBS32129B	80 µl of (±)12(13)-DiHOME (1 µg/ml).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS32129C	80 µl of HRP-(±)12(13)-DiHOME conjugate (100x).	May be stored for up to 6 months at 2-8 °C.
Assay Diluent	TBS3000D	25 ml of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 1 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

Sample preparation for ELISA assay

The sample to be tested should be collected according to accepted sampling techniques.

ELISA Procedures

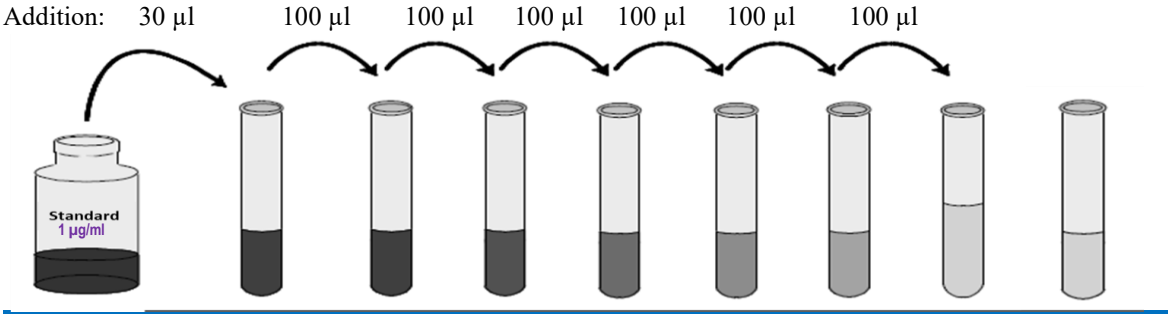
Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer. *(If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).*

(±)12(13)-DiHOME Standard Preparation: Label test tubes as #1 through #7. Pipet 270 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below.**

1. Add 30 µL of the (±)12(13)-DiHOME Standard stock solution (1 µg /mL) to tube #1 (100 ng/mL) and mix.
2. Make 3x serial dilutions of the standard using the Tube#1(100 ng/mL standard solution) from Tube #2 through #7 with sequential transfer of 100 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 100, 33.33, 11.11, 3.70, 1.23, 0.41 and 0.14 ng/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer(µL)	270	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	30	100	100	100	100	100	100	0
Final Conc (ng/ml)	100	33.33	11.11	3.70	1.23	0.41	0.14	0

Assay Procedures:

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 45min**.
3. Aspirate each well, and wash for 3 times by filling each well with 200 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
5. Add 100 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Quantitative Calculation of (±)12(13)-DiHOME Concentration

a) Calculate B/B0

Dividing average absorbance of each standard and sample (B) by absorbance of the standard 0 ng/mL (±)12(13)-DiHOME concentration, B0) to obtain percentage absorbance as below:

Percentage absorbance (%) = 100% x (B/B0)

B: Average absorbance of a standard or sample

B0: Average absorbance of 0 ng/mL standard

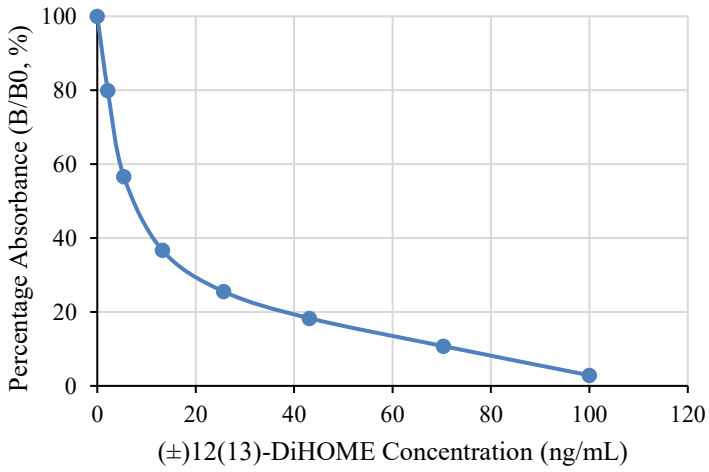
b) A standard curve Calculation: A standard curve is obtained by graphing the percentage absorbance of standards (Y axis) versus their corresponding concentration (X axis), and sample concentration can be read from this standard curve. Alternatively, (±)12(13)-

DiHOME concentration in the samples can be calculated with regression equation correlating percentage absorbance to (±)12(13)-DiHOME concentration.

Typical Data

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

Fig 3. (±)12(13)-DiHOME Standard Curve



Technical Assistance

For ordering or technical assistance regarding this kit, or for additional information about TribioScience products, please email: support@tribioscience.com or call (408) 498-0197 or 833-697-8998 (Toll Free).

Relative Products

Human p-Tau-217 ELISA (TBS3293)
Human p-Tau-181 ELISA (TBS3294)
Human Total Tau ELISA (TBS3295)
Human p-Tau-231 ELISA (TBS3296)
Human AD7c NTP (TBS3297)
Human Amyloid ß40 ELISA (TBS3298)
Human NF-L ELISA (TBS32101)
Human Total Amyloid ß ELISA (TBS32104)
Human UCHL1/PGP9.5 ELISA (TBS32107)
Human Gamma H2AX ELISA (TBS3265)
Human H2AX ELISA (TBS3266)
Human IL-4 ELISA (TBS3221)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)

For research use only.