New standard storage temperature

15(S)-HETE ELISA kit

Catalog No. ADI-901-051

480 Well (5 by 96 Well) Kit

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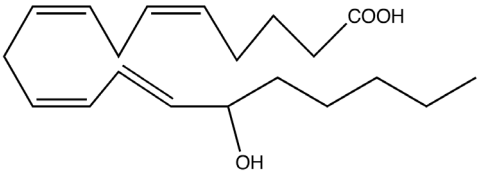
Description

The 15(S)-HETE ELISA kit is a competitive immunoassay for the quantitative determination of 15(S)-HETE in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a polyclonal antibody to 15(S)-HETE to bind, in a competitive manner, the 15(S)-HETE in the standard or sample or an alkaline phosphatase molecule which has 15(S)-HETE covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated is read on a microplate reader at 405nm. The intensity of the bound yellow color is inversely proportional to the concentration of 15(S)-HETE in either standards or samples. The measured optical density is used to calculate the concentration of 15(S)-HETE. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

15-hydroxyeicosatetraenoic acid [15(S)-HETE] is the major hydroxy derivative of arachidonic acid when acted upon by 15-lipoxygenase (15-LOX). It is also the primary monohydroxy acid synthesized by the lipoxygenase activity of Cyclooxygenase-1. Aspirin-mediated acetylation of the COX-1 enzyme results in 15(R)-HETE[3]. Blood platelets, peripheral leukocytes, vascular smooth muscle and other cell types produce Type-1 15-LOX while prostate, lung, skin and cornea tissues produce Type-2[4,5]. 15-HETE has been proposed to act as a paracrine regulator of smooth muscle and lung neutrophil recruitment due in part to its incorporation into tracheal epithelium at the *sn*-2 position of phosphatidylinositol[6]. The phosphoinositol modification in turn is thought to affect signal transduction and the regulation of intracellular calcium[7,8]. Interleukin-4 has been shown to regulate 15(S)-HETE expression and incorporation into cellular phospholipids[9,10]. 15(S)-HETE binds to actin and the alpha-subunit of mitochondrial ATP synthase suggesting a more direct method in regulating some physiological activities[11]. Increased levels of 15(S)-HETE are associated with asthma, rhinitis, chronic paranasal sinusitis and rheumatoid arthritis[12-14].

15(S)-HETE



Precautions

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1. Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.
2. Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.
3. The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.

4. We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
5. The 15(S)-HETE Standard provided, Catalog No. 80-0404, is supplied in ethanolic buffer at a pH optimized to maintain 15(S)-HETE integrity. Care should be taken handling this material because of the known and unknown effects of eicosanoids.

Materials Supplied

1. **Goat anti-Rabbit IgG Microtiter Plate, Five Plates of 96 Wells, Catalog No. 80-0060**
   Plates using break-apart strips coated with goat antibody specific to rabbit IgG.
2. **15(S)-HETE ELISA Conjugate, 25 mL, Catalog No. 80-0818**
   A blue solution of alkaline phosphatase conjugated with 15(S)-HETE.
3. **15(S)-HETE ELISA Antibody, 25 mL, Catalog No. 80-0817**
   A yellow solution of a rabbit polyclonal antibody to 15(S)-HETE.
4. **Assay Buffer Concentrate, 27 mL, Catalog No. 80-0011**
   Tris buffered saline containing proteins and sodium azide as preservative.
5. **Wash Buffer Concentrate, 100 mL, Catalog No. 80-1287**
   Tris buffered saline containing detergents.
6. **15(S)-HETE Standard, 0.5 mL x 3, Catalog No. 80-0404**
   A solution of 200,000 pg/mL 15(S)-HETE.
7. **pNpp Substrate, 100 mL, Catalog No. 80-0076**
   A solution of p-nitrophenyl phosphate in buffer. Ready to use.
8. **Stop Solution, 30 mL, Catalog No. 80-0248**
   A solution of trisodium phosphate in water. Keep tightly capped. Caution: **Caustic.**
9. **15(S)-HETE Assay Layout Sheet, 1 each, Catalog No. 30-0103**
10. **Plate Sealers, 10 each, Catalog No. 30-0012**

Storage

All components of this kit, **except the Conjugate and Standard**, are stable at 4°C until the kit's expiration date. The Conjugate and Standard **must** be stored frozen at -20°C.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 µL and 1,000 µL.
3. Repeater pipets for dispensing 50 µL and 200 µL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A 37°C incubator.
7. Absorbent paper for blotting.
8. Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.

Sample Handling

The 15(S)-HETE ELISA kit is compatible with 15(S)-HETE samples in a wide range of matrices. Samples diluted sufficiently into Assay Buffer can be read directly from the standard curve. Please refer to the Sample Recovery recommendations on page 11 for details of suggested dilutions. However, the end user must verify that the recommended dilutions are appropriate for their samples. **Samples containing rabbit IgG may interfere with the assay.**

Culture fluids, serum and plasma are suitable for use in the assay. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer. There will be small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of 15(S)-HETE in the appropriate matrix.

Plasma, serum and urine samples may need to be extracted. Some samples normally have very low levels of 15(S)-HETE present and extraction may be necessary for accurate measurement. Tissue samples should be extracted. A suitable extraction procedure is outlined below:

Materials Needed

1. 15(S)-HETE Standard to allow extraction efficiency to be accurately determined.
2. 2M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate.
3. 200 mg C_{18} Reverse Phase Extraction Columns.

Procedure

1. Acidify the plasma, urine or tissue homogenate by addition of 2M HCl to pH of 3.5. Approximately 50 µL of HCl will be needed per mL of plasma. Allow to sit at 4°C for 15 minutes. Centrifuge samples in a microcentrifuge for 2 minutes to remove any precipitate.
2. Prepare the C_{18} reverse phase column by washing with 10mL of ethanol followed by 10 mL of deionized water.
3. Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5 mL/minute. Wash the column with 10 mL of water, followed by 10 mL of 15% ethanol, and finally 10 mL hexane. Elute the sample from the column by addition of 10 mL ethyl acetate.
4. If analysis is to be carried out immediately, evaporate samples under a stream of nitrogen. Add at least 250 µL of Assay Buffer to the dried samples. Vortex well then allow to sit for five minutes at room temperature. Repeat twice more. If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at -80°C until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running assay and reconstitute as above.

Please refer to references 15-18 for details of extraction protocols.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
8. **Care must be taken to minimize contamination by endogenous alkaline phosphatase.** Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
9. **Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**

Reagent Preparation

1. Assay Buffer

Just before use, prepare the assay buffer by diluting 10 mL of the supplied Assay Buffer Concentrate with 90 mL of deionized water. Discard unused buffer or add up to 0.09% sodium azide (w/v) for storage.

2. 15(S)-HETE Standard

Allow the 200,000 pg/mL 15(S)-HETE standard solution to warm to room temperature. Label five 12 x 75 mm glass tubes #1 through #5. Pipet 1 mL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1. Pipet 750 µL of standard diluent into tubes #2 through #5. Remove 100 µL of standard diluent from tube #1. Add 100 µL of the 200,000 pg/mL standard to tube #1. Vortex thoroughly. Add 250 µL of tube #1 to tube #2 and vortex thoroughly. Add 250 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 and #5.

The concentration of 15(S)-HETE in tubes #1 through #5 will be 20,000, 5,000, 1,250, 312.5 and 78.12 pg/mL respectively. See 15(S)-HETE Assay Layout Sheet for dilution details.

Diluted standards should be used within 60 minutes of preparation.

3. Wash Buffer

Prepare the Wash Buffer by diluting 5 mL of the supplied concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 C.
2. Pipet 100 µL of standard diluent (Assay Buffer or Tissue Culture Media) into the NSB and the Bo (0 pg/mL Standard) wells.
3. Pipet 100 µL of Standards #1 through #5 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Pipet 50 µL of Assay Buffer into the NSB wells.
6. Pipet 50 µL of blue Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipet 50 µL of yellow Antibody into each well, except the Blank, TA and NSB wells.

NOTE: Every well used should be **Green** in color except the NSB wells which should be **Blue**. The Blank and TA wells are empty at this point and have no color.

8. Incubate the plate for 18-24 hours at 4°C. The plate should be covered with the plate sealer provided.
9. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 2 more times for a total of **3 Washes.**
10. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 5 µL of the blue Conjugate to the TA wells.
12. Add 200 µL of the pNpp Substrate solution to every well. Incubate at 37°C for 1 hour without shaking. The plate should be covered with the provided plate sealer.
13. Add 50 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.
14. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Calculation of Results

Several options are available for the calculation of the concentration of 15(S)-HETE in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of 15(S)-HETE can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:
   Average Net OD = Average Bound OD - Average NSB OD

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:
   $$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

3. Plot Percent Bound versus Concentration of 15(S)-HETE for the standards. Approximate a straight line through the points. The concentration of 15(S)-HETE in the unknowns can be determined by interpolation.

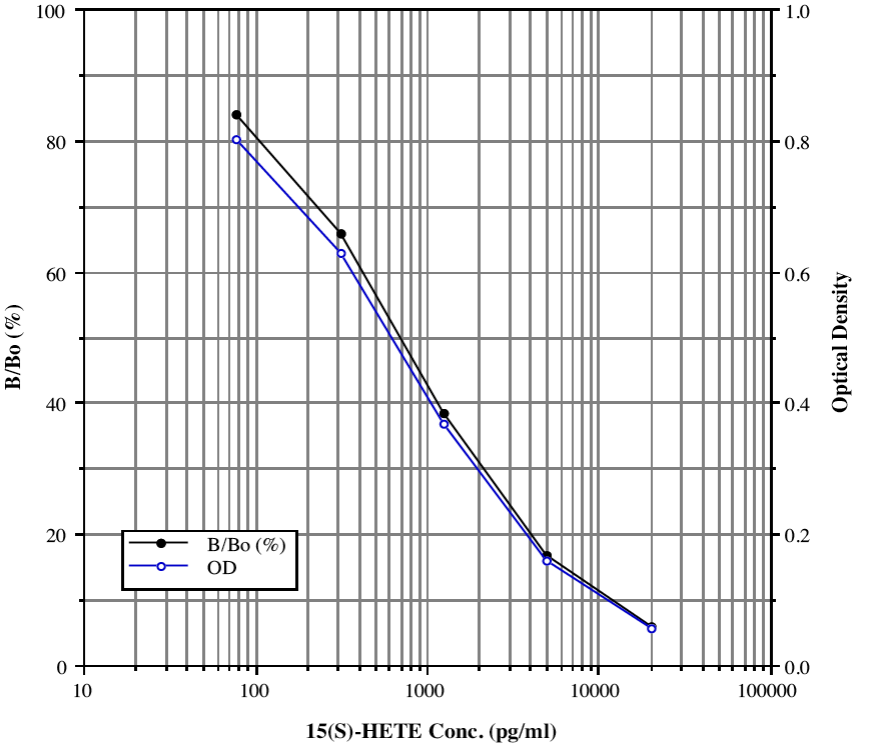
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results.

Sample	Mean OD(-Blank)	Average Net OD	Percent Bound	**15(S)-HETE (pg/mL)**
Blank OD	(0.084)			
TA	0.597	0.598		
NSB	0.083	-0.001		
Bo	0.954	0.955	100.0%	**0**
S1	0.056	0.057	5.9%	**20,000**
S2	0.159	0.160	16.8%	**5,000**
S3	0.367	0.368	38.6%	**1,250**
S4	0.628	0.629	65.9%	**312.5**
S5	0.802	0.803	84.1%	**78.12**
Unknown 1	0.229	0.230	24.1%	**2,872**
Unknown 2	0.580	0.581	60.8%	**410**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate 15(S)-HETE concentrations; each user must run a standard curve for each assay.



Typical Quality Control Parameters

Total Activity Added	=	0.598 x 10 = 5.98
%NSB	=	-0.02%
%Bo/TA	=	15.97%
Quality of Fit	=	1.000 (Calculated from 4 parameter logistic curve fit)
20% Intercept	=	3,819 pg/mL
50% Intercept	=	710 pg/mL
80% Intercept	=	117 pg/mL

Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[19].

Sensitivity

Sensitivity was calculated by determining the average optical density bound for fifteen (15) wells run in Bo, and comparing to the average optical density for fifteen (15) wells run with Standard #5. The detection limit was determined as the concentration of 15(S)-HETE measured at two (2) standard deviations from the zero along the standard curve.

Average Optical Density for the Bo =	1.008 $\pm$ 0.066 (6.60%)
Average Optical Density for the Standard #5 =	0.859 $\pm$ 0.049 (5.73%)
Delta Optical Density (0-78.12 pg/mL) =	0.149
2 SD's of the Zero Standard = 2 x 0.066 =	0.132

Sensitivity = $\frac{0.132}{0.149}$ x 78.12 pg/mL = **69.21 pg/mL**

Linearity

A sample containing 9,923 pg/mL 15(S)-HETE was serially diluted 5 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual 15(S)-HETE concentration versus measured 15(S)-HETE concentration.

The line obtained had a slope of 0.8184 and a correlation coefficient of 0.99927.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of 15(S)-HETE and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of 15(S)-HETE in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of 15(S)-HETE determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	15(S)-HETE (pg/mL)	Intra-assay %CV	Inter-assay %CV
Low	181	15.8	
Medium	853	9.8	
High	3929	3.4	
Low	192		19.1
Medium	730		8.9
High	4,335		6.1

Cross Reactivities

The cross reactivities for a number of related eicosanoid compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 500,000 to 5 pg/mL. These samples were then measured in the 15(S)-HETE assay, and the measured 15(S)-HETE concentration at 50% B/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
15(S)-HETE	100%
5,15-diHETE	1.0%
8,15-diHETE	1.0%
13(S)-HODE	0.6%
5-HETE	0.1%
PGB_2	0.1%
PGD_2	0.1%
$PGF_{2\alpha}$	0.1%
12(S)-HETE	<0.05%
12(R)-HETE	<0.05%
Arachidonic Acid	<0.05%
PGE_2	<0.05%
Linoleic Acid	<0.05%

Sample Recoveries

Please refer to pages 4 and 5 for Sample Handling recommendations and Standard preparation.

15(S)-HETE concentrations were measured in a variety of different samples including tissue culture media, human urine, porcine EDTA plasma and porcine serum. For all of the samples, 15(S)-HETE was spiked into the undiluted samples which were diluted with the kit Assay Buffer and then assayed in the kit. The following results were obtained:

Sample	% Recovery*	Recommended Dilution*
Tissue Culture Media	100.3	None
Human Urine	89.6	≥1:8
Porcine EDTA Plasma	105.4	1:4 - 1:32
Porcine Serum	95.9	≥1:16

* See Sample Handling on page 4 for details.

References

1. T. Chard, in "An Intro. to Radioimmunoassay & Related Tech.", (1990), 4th Ed., Elsevier, Amsterdam.
2. P. Tijssen, in "Practice and Theory of Enz. Immunoassays", (1985), Elsevier, Amsterdam.
3. E.D. Thuresson, et al., J. Biol. Chem., (2000), 275/12:8501.
4. S.B. Shappel, et al., Am. J. Path., (1999), 155/1:235.
5. J. Martyn Bailey, et al., J. Lipid Res., (1982), 24:1419.
6. S.E. Alpert and R.W. Walenga, Am. J. Respir. Cell Mol. Biol., (1993), 8:273.
7. R.J. Smith et al., Proc. Natl. Acad. Sci. USA, (1993), 90:7270.
8. S.E. Alpert et al., Am, J. Physiol., (1999), 277:L457.
9. S.P. Jayawickreme, et al., Am. J. Physiol., (1999), 276:L596.
10. M. Profita, et al., Am. J. Respir. Cell Mol. Biol., (1999), 20:61.
11. L.T. Kang, et al., Biochim Biophys Acta, (1999), 1438/3:388.
12. M. Profita, et al., J. Allergy Clin. Immuno., (2000), 105/4:
13. Y.J. Jang, et al., Acta Otolaryngol., (2000), 120/4:531.
14. B. Liagre, et al., J. Rheumatol., (1999), 26/5:1044.
15. K. Green, et al., Anal. Biochem., (1973), 54, 434.
16. J. Frolich, et al., J. Clin. Invest., (1975), 55, 763.
17. J.E. Shaw & P.W. Ramwell, Meth. Biochem. Anal., (1969), 17, 325.
18. K. Green et al., Adv. Prostaglandin & Thromboxane Res., (1978), 5, 15.
19. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

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TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending.

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