Package Insert

Lipoxin A_4 ELISA

Catalog Number: LA439-K01
96 Wells
For Research Use Only
v. 2.0 (08.8.22)

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INTRODUCTION

Lipoxin A_4 (LXA_4) is a biologically active lipoxygenase interaction product derived from arachidonic acid. Arachidonic acid is first oxygenated by 15-lipoxygenase to form 15-HETE which is converted by 5-lipoxygenase and epoxide hydrase to generate LXA_4. LXA_4 stimulates leukocyte chemotaxis without aggregation and inhibits natural killer cell cytotoxicity. It also provokes contraction of parenchymal strips and stimulates microvascular changes. Recent findings indicate that LXA_4 inhibits leukocyte-dependent inflammation. Determination of LXA_4 level may provide new understanding of the role of LXA_4 in basic cellular reactions and in pathophysiology of inflammation and other disease processes.

PRINCIPLES OF PROCEDURE

Lipoxin A_4 ELISA (Enzyme-Linked ImmunoSorbent Assay) is for the quantitative analysis of Lipoxin A_4 levels in biological fluid. This test kit operates on the basis of competition between the enzyme conjugate and the LXA_4 in the sample for a limited number of antibody binding sites.

The sample or standard solution is first added to the microplate. Next, the diluted enzyme conjugate is added and the mixture is shaken and incubated at room temperature for one hour. During the incubation, competition for binding sites is taking place. The plate is then washed removing all the unbound material. The bound enzyme conjugate is detected by the addition of substrate that generates an optimal color after 30 minutes. Quantitative test results may be obtained by measuring and comparing the absorbance reading of the wells of the samples against the standards with a microplate reader at 650 nm. The extent of color development is inversely proportional to the amount of LXA_4 in the sample or standard. For example, the absence of LXA_4 in the sample will result in a bright blue color, whereas the presence of LXA_4 will result in decreased or no color development.

MATERIALS PROVIDED

Component	Description	Volume	Storage
EIA Buffer	Buffer used to dilute the Conjugate and LXA_4 Standards.	30 mL	4°C
10x Wash Buffer	Buffer used to wash the plate prior to color development.	20 mL	4°C
TMB Substrate	TMB substrate used for color development.	20 mL	4°C
5x Extraction Buffer	Buffer used to dilute extracted and non-extracted samples.	30 mL	4°C
LXA_4-HRP Conjugate	Lyopholized LXA_4 horseradish peroxidase conjugate.	2 vials	-20°C
LXA_4 Standard	1 µg/mL LXA_4 standard solution.	100 µL	4°C
Coated Plate	96-well microplate coated with rat anti-LXA_4 antibody.	1 plate	4°C

MATERIALS NEEDED BUT NOT PROVIDED

1. Microplate reader with a 450 nm or 650 nm filter
2. Adjustable micropipettes (10 – 1000 µL) and tips
3. Deionized water
4. Plate cover or plastic film
5. Test tubes
6. 1 N HCl (optional)

EXTRACTION MATERIALS

1. 1 N HCl
2. Methanol
3. C_{18} Sep-Pak® Light Column (Waters® Corporation #23501)
4. Hexane
5. Methyl Formate
6. Nitrogen Gas
7. Centrifuge

STORAGE

1. Store the components of this kit at the temperatures specified on the labels.
2. Unopened reagents are stable until the indicated kit expiration date.
3. Concentrated, reconstituted conjugate has a shelf life of at least two weeks when stored properly.
4. Desiccant bag must remain in foil pouch with unused strips. Keep pouch sealed when not in use to maintain a dry environment. Remove excess air before sealing.

WARNINGS AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.

PROCEDURAL NOTES

1. Ensure that the conjugate is completely reconstituted before use. Each vial, when reconstituted, provides sufficient reagent to perform 64 wells (8 strips). If more than 64 assays are to be run, reconstitute both vials and pool the reconstituted conjugate.
2. To minimize errors in absorbance measurements due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel prior to inserting into the plate reader.

SAMPLE PREPARATION

- Plasma, serum, urine and tissue culture need to be extracted.

EXTRACTION PROTOCOL

1. Dilute 100 µL of sample with 200 µL of methanol, and then dilute that volume with 1.5 mL of water.
2. Acidify 1 mL of biological fluid to pH 3.5 with 1 N HCl (about 150 µL for plasma) and vortex.
3. For tissue, homogenize it in ethanol (5 mL/g). Centrifuge the homogenate for five minutes. Collect the supernatant in a clean tube. Dilute 1 mL of the supernatant with 5 mL of deionized water and acidify to pH 3.5 with 1 N HCl.

4. Precondition the C_{18} Sep-Pak® Light column by washing the column with 2 mL of methanol followed by 2 mL of deionized water.
5. Apply the above sample into the column and adjust the flow rate to 1 mL per minute. Reducing the flow rate to 0.5 mL per minute may increase extraction efficiencies.
6. Wash the column with 5 mL of water followed by 5 mL of hexane.
7. The $LXA_4$is eluted by 2 mL of methyl formate.
8. Evaporate the methyl formate eluate with a stream of nitrogen gas.
9. Resuspend the residue with 1 mL of diluted Extraction Buffer. The residue may be dissolved in less than 1 mL if the concentration is suspected to be low (<0.1 ng per mL).

REAGENT PREPARATION

1. **5x Extraction Buffer:** Dilute the appropriate amount to 1x with deionized water prior to use.
2. **10x Wash Buffer:** Add 20 mL of 10x Wash Buffer to 180 mL of deionized water prior to use.
3. **LXA_4-HRP Conjugate:** Reconstitute by adding 75 µL of deionized water to each vial. Dilute 110 µL of Conjugate into 5.5 mL total volume of EIA Buffer.

STANDARD CURVE PREPARATION

The LXA_4 Standard is provided as a 1 µg/mL stock solution. Use the following tables to dilute a set of standard stock solutions and construct an eight-point standard curve.

Table 1: Standard Stock Preparation

Standard	LXA_4 Conc. (ng/mL)	Vol. of EIA Buffer (µL)	Transfer Vol. (µL)	Final Vol. (µL)
A	1000	-	Provided	80
B	20	980	20 µL of A	800
C	2	1800	200 µL of B	1800
D	0.2	1800	200 µL of C	2000

Table 2: Standard Curve Preparation

Standard	LXA_4 Conc. (ng/mL)	Vol. of EIA Buffer (µL)	Vol. of Stock C (µL)	Vol. of Stock D (µL)
S_0	0	1000	-	-
S_1	0.02	900	-	100
S_2	0.04	800	-	200
S_3	0.1	500	-	500
S_4	0.2	-	-	1000
S_5	0.4	800	200	-
S_6	0.8	600	400	-
S_7	2.0	-	1000	-

ASSAY PROCEDURE

1. Add 50 µL of Standards or Samples (may require diluting) to the corresponding wells on the microplate in duplicate. See **Scheme I** for a sample plate layout.
2. Add 50 µL of diluted LXA_4-HRP Conjugate to each well. Incubate at room temperature for one hour.
3. Wash the plate three times with 300 µL of diluted Wash Buffer per well. Wash 5 times if using an automated plate washer.
4. Add 150 µL of TMB Substrate to each well. Incubate at room temperature for 30 minutes.
5. Read the plate at 650 nm.

Alternately, the color reaction can be stopped after 10-15 minutes by adding 50 µL of 1 N HCl and read at 450 nm.

NOTE: If accounting for substrate background, use 2 wells as blanks (BLK) with only 150 µL TMB Substrate in the wells. Subtract the average of these absorbance values from the absorbance values of the wells being assayed.

Scheme I: Sample Plate Layout

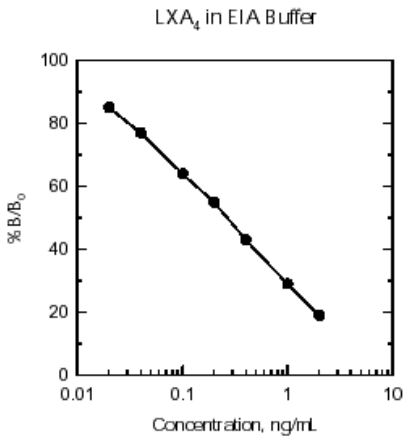
	1	2	3	4	5	6	7	8	9	10	11	12
A	S_0	S_0	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}
B	S_1	S_1	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}
C	S_2	S_2	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
D	S_3	S_3	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
E	S_4	S_4	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
F	S_5	S_5	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
G	S_6	S_6	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
H	S_7	S_7	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	BLK	BLK

CALCULATIONS

1. After the substrate background has been subtracted from all absorbance values, average all of your duplicate well absorbance values.
2. The average of your two S_0 values is now your B_0 value. (S_1 now becomes B_1, etc.)
3. Next, find the percent of maximal binding (%B/B_0 value). To do this, divide the averages of each standard absorbance value (now known as B_1 through B_7) by the B_0 absorbance value and multiply by 100 to achieve percentages.
4. Graph your standard curve by plotting the %B/B_0 for each standard concentration on the y-axis against concentration on the x-axis. Draw a curve by using a curve-fitting routine (i.e. 4-parameter or linear regression).
5. Divide the averages of each sample absorbance value by the B_0 value and multiply by 100 to achieve percentages.

6. Using the standard curve, the concentration of each sample can be determined by comparing the %B/ B_0 of each sample to the corresponding concentration of standard.
7. If the samples were diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

Figure 1: Typical Standard Curve



CROSS REACTIVITY

Lipoxin A_4	100.0%	12-HETE	<0.10%
15-epi- Lipoxin A_4	24%	Leukotriene B_4	<0.01%
5(S)6(R)-Di-HETE	5.00%	Leukotriene C_4	<0.01%
Lipoxin B_4	1.00%	Leukotriene D_4	<0.01%
15-HETE	0.10%	Leukotriene E_4	<0.01%
5-HETE	<0.10%		

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.