Package Insert

15-epi-Lipoxin A_4 ELISA

Catalog Number: 15E39-K01
96 Wells
For Research Use Only
v. 2.0 (08.03.2022)

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INTRODUCTION

15-epi-Lipoxin A_4 (15-epi-LXA_4) is an aspirin-triggered eicosanoid believed to be involved in the positive attributes of aspirin therapy for heart, cancer, and human immunodeficiency virus patients. During inflammation, neutrophils are activated. 15-epi-LXA_4, when administered in vivo, inhibits neutrophil activation and dampens inflammation (Takano, 1997). 15-epi-LXA_4 is naturally formed in the body via the following pathway:

Arachidonic Acid (AA) □ 15(R)-HETE □ 15-epi-Lipoxin A_4

Aspirin is thought to be involved in the acetylation of prostaglandin G/H synthase, which triggers the conversion of AA to 15(R)-HETE.

The development of this assay, which is both sensitive and selective for 15-epi-LXA_4, will be essential to researchers studying anti-inflammatory medications.

PRINCIPLE OF ASSAY

The 15-Epi-Lipoxin A4 ELISA Kit (Enzyme-Linked Immunosorbent Assay) for the quantitative analysis of 15-epi-Lipoxin A_4 levels in biological fluid. This test kit operates on the basis of competition between the enzyme conjugate and the 15-epi-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 15-epi-LXA_4 in the sample or standard. For example, the absence of 15-epi-LXA_4 in the sample will result in a bright blue color, whereas the presence of 15-epi-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 15-epi-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
15-epi-LXA_4-HRP Conjugate	Lyopholized 15-epi-LXA_4 horseradish peroxidase conjugate.	2 vials	-20°C
15-epi-LXA_4 Standard	1 µg/mL 15-epi-LXA_4 standard solution.	50 µL	4°C
Coated Plate	96-well microplate coated with rabbit anti-15-epi-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. Ethanol
3. C_{18} Sep-Pak® Light Column (Waters® Corporation #23501)
4. Petroleum Ether
5. Methanol
6. Nitrogen Gas
7. Centrifuge

STOARAGE

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

- Use aseptic technique when opening and dispensing reagents.
- 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

- 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.
- 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

a) Urine and tissue culture supernatant can be assayed directly by diluting them with Extraction Buffer.
b) Dilute specimens may require extraction in order to concentrate 15-epi-LXA_4.
c) Plasma and most other mediums will need to be extracted.

EXTRACTION PROTOCOL

1. Acidify 1 mL of biological fluid to pH 3.5 with 1 N HCl (about 150 µL for plasma) and vortex.
2. 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.
3. Precondition the C_{18} Sep-Pak® Light column by washing the column with 2 mL of methanol followed by 2 mL of deionized water.
4. 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. Some samples may clog the column. These samples may be diluted 1:5 in water to improve the flow rate.
5. Wash the column with 5 mL of water followed by 5 mL of petroleum ether.
6. The 15-epi-$LXA_4$is eluted by 2 mL of methyl formate.
7. Evaporate the methyl formate eluate with a stream of nitrogen gas.
8. 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. **15-epi-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 15-epi-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	15-epi-LXA_4 Conc. (ng/mL)	Vol. of EIA Buffer (µL)	Transfer Vol. (µL)	Final Vol. (µL)
A	1000	-	Provided	40
B	20	490	10 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	15-epi-LXA_4 Conc. (ng/mL)	Vol. of EIA Buffer (µL)	Vol. of Stock C (µL)	Vol. of Stock D (µL)
S0	0	1000	-	-
S1	0.02	900	-	100

S2	0.05	750	-	250
S3	0.1	500	-	500
S4	0.2	-	-	1000
S5	0.5	750	250	-
S6	1.0	500	500	-
S7	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 15-epi-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.
6. 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	S0	S0	U1	U1	U9	U9	U17	U17	U25	U25	U33	U33
B	S1	S1	U2	U2	U10	U10	U18	U18	U26	U26	U34	U34
C	S2	S2	U3	U3	U11	U11	U19	U19	U27	U27	U35	U35
D	S3	S3	U4	U4	U12	U12	U20	U20	U28	U28	U36	U36
E	S4	S4	U5	U5	U13	U13	U21	U21	U29	U29	U37	U37
F	S5	S5	U6	U6	U14	U14	U22	U22	U30	U30	U38	U38
G	S6	S6	U7	U7	U15	U15	U23	U23	U31	U31	U39	U39
H	S7	S7	U8	U8	U16	U16	U24	U24	U32	U32	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 S0 values is now your B0 value. (S1 now becomes B1, etc.)
3. Next, find the percent of maximal binding (%B/B0 value). To do this, divide the averages of each standard absorbance value (now known as B1 through B7) by the B0 absorbance value and multiply by 100 to achieve percentages.
4. Graph your standard curve by plotting the %B/B0 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 B0 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/ B0 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.

CROSS REACTIVITY

15-epi-Lipoxin A_4	100.0%	5(S)-HETE	<0.01%
Lipoxin A_4	3%	12(S)-HETE	<0.01%
15(R)-HETE	0.8%	15(S)-HETE	<0.01%

REFERENCES

1. Takano, T., *et. al.*, (1997) *J. Exp. Med.* 185(9): 1693-1704
2. Takano, T., *et. al.*, (1998) *J. Clin. Invest.* 101: 819-826

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