Shanghai Coon Koon Biotech Co., Ltd

Room 503, 2799 Huqingping Highway, Shanghai, China

www.coonkoonbio.com

+86 15001964983

Mouse Lipoxin A4 (LXA4) ELISA Kit

Cat No:CK-bio-16528

Standard Curve Range: 10pg/mL -480pg/mL

Sensitivity: 1.0pg/mL

Expiration date： six months .

Storage： 2-8℃.

For samples: Serum, plasma, cell culture supernatants, body fluid and tissue homogenate

When stored at 2 -8 °C unopened reagents will retain reactivity until expiration date.

Opened reagents must be stored at 2 -8 °C.

OPERATION MANUAL

Read this manual carefully before using. The ELISA kit is based on the principle of double antibody sandwich technology.

And the ELISA kits only be used for research purposes, not for medical diagnosis.

Reagent preparation: Bring all reagents to room temperature before using.

Intended Use

For the quantitative determination of Mouse Lipoxin A4(LXA4) concentrations in serum, plasma, saliva, urine, tissue homogenate, cell culture supernates and other biological fluids.

Test Principle

The kit was used to test the level of Mouse Lipoxin A4(LXA4), based on the principle of double antibody sandwich technology enzyme linked immunosorbent assay (ELISA).

Add Standard and Sample to the wells that pre-coated with objective antibody, then add HRP-Conjugate reagent to form an immune complex, incubation, by incubation and washing, removal of unbound enzyme, and then add the substrate A and B, then the solution will turn blue and finally change into yellow at the effect of acid. The color depth or light was positively correlated with the concentration of Lipoxin A4 (LXA4).

Precautions

1.Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.

2.It is highly recommended to use the remaining reagents within 1 month before the deadline. For the expiration date, please refer to the label on the kit box. All components are stable before this expiration date.Do not use kit components beyond their expiration date.

3.Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.

4.Use only deionized or distilled water to dilute reagents.

5.Each steps add sample, should use sampler, and often proofread the accuracy to avoid the test error. Use fresh disposable pipette tips for each transfer to avoid contamination.

6.Test should strict accordance with the instructions of the operation, the test results must be determined by the microplate reader.

7.Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.

8.Do not mix acid and sodium hypochlorite solutions.

9.Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from Rat blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

10.All samples should be disposed of in a manner that will inactivate viruses.

11.Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste

should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

12.Substrate Solution is easily contaminated. If bluish prior to use, do not use. Substrate B is sensitive to light and avoid prolonged exposure to light.

MATERIALS PROVIDED WITH THE KIT

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

Reagents components		96 determinations	48 determinations
1.	Microelisa stripplate	12*8strips	12*4strips
2.	Standard A	0pg/mL	0pg/mL
3.	Standard B	30pg/mL	30pg/mL
4.	Standard C	60pg/mL	60pg/mL
5.	Standard D	120pg/mL	120pg/mL
6.	Standard E	240pg/mL	240pg/mL
7.	Standard F	480pg/mL	480pg/mL
8.	Sample Diluent	6.0ml	3.0ml
9.	HRP-Conjugate reagent	10.0ml	5.0ml
10.	20X Wash solution	25ml	15ml
11.	Chromogen Solution A	6.0ml	3.0ml
12.	Chromogen Solution B	6.0ml	3.0ml
13.	Stop Solution	6.0ml	3.0ml

14.	Closure plate membrane	2	2
15.	User manual	1	1
16.	Sealed bags	1	1

Note: Standard (A → F) concentration was followed by: 0pg/mL ,30pg/mL ,60pg/mL ,

120pg/mL ,240pg/mL ,480pg/mL.

Materials required but not supplied

1.37 ℃ incubator

2.Microplate reader capable of measuring absorbance at 450 nm.

3.Precision pipettes to deliver 2 ml to 1 ml volumes.

4.100 ml and 1 liter graduated cylinders.

5. Distilled water,

6. Disposable test tube

7. Absorbent paper

8. Precision pipettes and disposable tip

Specimen Requirements

1.**Serum:** Allow the serum to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

2.**Blood plasma**: In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as anti coagulation. Add EDTA or sodium citrate and mix them for 10-20 minutes. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

3.**Urine**: Collect by sterile tube. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow the procedures above-mentioned.

4.**Cell culture supernatant**: Collect by sterile tubes when examining secrete components. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (PH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

5.**Tissue sample**: Incise sample and weigh up. Add a certain amount of PBS (PH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8℃. Add a certain amount of PBS (PH 7.4) and then homogenize the sample thoroughly by hand or homogenizer. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

Note: 1.Samples to be used within 5 days may be stored at 2-8℃, otherwise samples must be stored at -20℃ (≤1month) or -80℃ (≤2 months) to avoid loss of bioactivity and avoid contamination.

2.Sample hemolysis will influence the result, so the samples should be centrifuged adequately and no hemolysis or granule was allowed.

3.When performing the assay, bring samples to room temperature.

Samples containing NaN3 can't be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).

4.After collecting the sample, extraction should be immediately carried out in accordance with related documents. After extraction, experiment should be conducted immediately as well. Otherwise, keep the sample at -20℃. Avoid repeated freeze-thaw cycles.

Washed plate method

1.Hand-washed plate method: get rid of the liquid within the ELISA plate; in the experimental bench paved a few layers of absorbent paper, pat hard the ELISA plate several times downward; the diluted washing solution at least 0.35ml inject into the well, soaking 1-2 minutes. Repeat this process several times as needed.

2. Automatic plate washing: If you have automatic washing machine, Should be skilled use, and then used in the formal experiment process.

Assay procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.

2. Add standard: Set Standard wells, testing sample wells. Add standard 50µl to standard well.

3. Add Sample: ①Add Sample 10µl to testing sample well, then add sample diluent 40µl to testing sample well; Blank well doesn't add anything.

② Add 100µl of HRP-conjugate reagent to each well(Standard wells and testing sample wells), then cover it with seal plate membrane, gently shake and mix for 60 minutes at 37 ° C incubation.

4.Preparation of washing solution: Dilute the washing concentration (20X) with distilled or deionized water for later use.

5.Washing by hand: carefully remove the sealing film, drain the liquid, dried up, each well filled with washing solution, put it aside for 1 min then drain the liquid, so repeat 5 times, pat dry. (Automatic washing: Each wells inject into the wash solution 350µL, soak 1min, wash plate 5 times.)

6. Color developing: firstly add 50µl chromogen solution A to each wells, then add 50µl chromogen solution B to each well as well. Shake gently to mix up. Incubate for 15 minutes at 37℃,away from light for color developing.

7.Stop: Add 50µl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment). If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8.Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450nm wavelength, which should be carried out within the 15 minutes after having added the stop solution.

9.According to standards' concentrations and the corresponding OD values, to calculate the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample.

Also can use related application software.

Summary of operating procedures

Prepare reagents, samples and standards

Add prepared samples and standards,and HRP, 37°C incubation for 60 minutes

Wash the plate 5 times, add chromogen solution A, B, 37°C developing color for 15 minutes

Add the stop solution

Read the OD value within 15 minutes

Calculate

Calculation of results

1. This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of

the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.

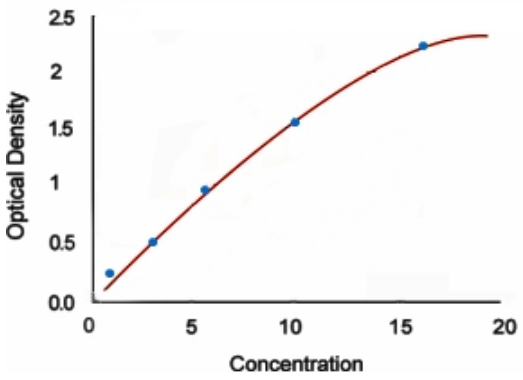
2. First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the zero standard before result interpretation. Construct the standard curve using graph paper or statistical software.

3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.

4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

5. Intra-assay CV(%) is less than 10% and Inter-assay CV(%) is less than 15%.

6.Standard curve : The following standard curve only for demonstration purposes, each standard curve should be generated with each assay.



1.Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

2.If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

3.If specimens generate values higher than the highest standard, dilute the specimens and repeat the assay.

FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS! PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!