ab287831 – Lipoxin A4 ELISA Kit

For in vitro quantitative measurement of Lipoxin A4
in serum, plasma, and other biological samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
https://www.abcam.com/ab287831

Storage and Stability

See Materials Supplied table below for storage conditions for individual kit components.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 wells x12 strips	-20°C
Reference Standard	2 vials	-20°C
Biotinylated Detection Ab (100x)	120 µl	-20°C
HRP Conjugate (100x)	120 µl	-20°C (Protect from light)
Standard & Sample Diluent	20 ml	4°C
Biotinylated Detection Antibody Diluent	14 ml	4°C
HRP Conjugate Diluent	14 ml	4°C
Wash Buffer (25X)	30 ml	4°C
Substrate Reagent	10 ml	4°C (Protect from light)
Stop Solution	10 ml	4°C
Plate Sealer	4	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Clean Eppendorf tubes for preparing standards or sample dilutions.

Reagent Preparation

- Bring all reagents to room temperature before use.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Wash Buffer (25X): Dilute 30 ml of Concentrated Wash Buffer with 720 ml of deionized or distilled water to prepare 750 ml of Wash Buffer.

Δ Note: *if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved.*

Biotinylated Detection Antibody working solution: Calculate the required amount (50 µl/well). Centrifuge the stock tube before use, dilute the 100x Concentrated Biotinylated Detection Antibody to 1x working solution with Biotinylated Detection Antibody Diluent.

HRP Conjugate working solution: Calculate the required amount before the experiment (100 µl/well). In preparation, slightly more than calculated should be prepared. Dilute the 100X Concentrated HRP Conjugate to 1X working solution with Concentrated HRP Conjugate Diluent.

Standard Preparation

- Centrifuge the standard at 10,000 x g for 1 min.
- Add 1.0 ml of Standard and Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 50 ng/ml.
- Make serial dilutions as needed. The recommended dilution gradient is as follows: 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0 ng/ml. Prepare 7 tubes, add 500 µl of Standard and Sample Diluent to each tube. Pipette 500 µl of the 50 ng/ml stock solution to the first tube and mix up to produce a 25 ng/ml working solution. Transfer 500 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay.

Sample Preparation

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 2-8°C before centrifugation for 15 min at 1000 x g at 2-8°C. Collect the supernatant. Blood collection tubes should be disposable and be endotoxin free.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000 x g at 2-8°C within 30 min of collection. Collect the supernatant. Hemolyzed samples are not suitable for ELISA assay!

Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000 x g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each $1x10^6$ cells, add 150-250 µl of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10 min at 1500 x g at 2-8°C. Remove the cell fragments; collect the supernatant to carry out the assay. Avoid repeated freeze-thaw cycles.

Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolyzed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01 M, pH 7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS volume (ml) = 1:9) with a glass homogenizer on ice. To further break down the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000 x g to get the supernatant.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000 x g at 2-8°C. Collect the supernatant to carry out the assay.

Assay Protocol

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Add the Standard working solution to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (50 µl for each well). Add the samples to the other wells (50 µl for each well). Immediately add 50 µl of Biotinylated Detection Antibody working solution to each well. Cover with the Plate sealer. Incubate for 45 min at 37°C.

   Δ Note: *solutions should be added to the bottom of the micro-ELISA plate well. Avoid touching the inside wall and causing foaming as much as possible.*

2. Aspirate or decant the solution from each well, add 350 µl of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

   Δ Note: *a microplate washer can be used in this step and other wash steps.*

3. Add 100 µl of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.

4. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 2.

5. Add 90 µl of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light.

   Δ Note: *the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.*

6. Add 50 µl of Stop Solution to each well.

   Δ Note: *Adding the stop solution should be done in the same order as the substrate solution.*

7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor. A standard curve must be run with each assay.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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