KAMIYA BIOMEDICAL COMPANY

Chicken Ex-FABP ELISA

For the quantitative determination of extracellular fatty acid binding protein (Ex-FABP) in chicken serum or plasma

Cat. No. KT-1887

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** Chicken Ex-FABP ELISA is an enzyme immunoassay for the quantitative determination of Ex-FABP in chicken serum or plasma. For research use only.

INTRODUCTION

Chicken extracellular fatty acid binding protein (Ex-FABP) is an antibacterial siderophore binding lipocalin that is expressed in serum and egg white. Proteomic studies indicate that it behaves as a positive acute phase protein; serum levels increase after lipopolysaccharide challenge. Our studies found plasma levels ranging from 0.5 to >10 µg/mL.

PRINCIPLE

The assay uses two chicken Ex-FABP antibodies; one for solid-phase immobilization (microtiter wells) and one, conjugated to horseradish peroxidase (HRP), for detection. Calibrators and diluted samples are incubated in the microtiter wells with HRP-conjugate for one hour. This results in Ex-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are washed and TMB is added and incubated for 20 minutes. If Ex-FABP is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of Ex-FABP is proportional to absorbance and is derived from a calibration curve.

COMPONENTS

- Ex-FABP antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 mL
- Ex-FABP calibrator stock (lyophilized)
- 20x Wash solution, 50 mL
- Diluent, 2 x 50 mL
- TMB, 11 mL
- Stop solution, 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettors and tips
- Distilled or de-ionized water
- Microcentrifuge tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

CALIBRATOR PREPARATION

1. The Ex-FABP stock is provided lyophilized. It consists of recombinant Ex-FABP diluted in a stabilizing protein matrix. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved **(the reconstituted calibrator remains stable for at least 1 day at room temperature but should be frozen at -20℃ after reconstitution if future use is intended).**
2. Label seven microcentrifuge tubes as 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/mL.

3. In the tube labeled 10 ng/mL prepare the 10 ng/mL calibrator as detailed on the stock vial label.
4. Dispense 250 µL of diluent into the tubes labeled 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/mL.
5. Prepare the 5 ng/mL calibrator by mixing 250 µL of the 10 ng/mL calibrator with 250 µL of diluent in the tube labeled 5 ng/mL.
6. Similarly prepare the remaining calibrators by two-fold serial dilution.

SAMPLE PREPARATION

To obtain values within the range of the calibration curve, we suggest that serum or plasma be diluted at least 1,000-fold (optimal dilution factors should be determined by the end user). A 1,000-fold dilution can be obtained as follows:
1. Dispense 190 µL and 490 µL of diluent into separate tubes.
2. Pipette and mix 10 µL of the plasma sample into the first tube containing 190 µL of diluent. This provides a 20-fold diluted sample.
3. Mix 10.0 µL of the 100-fold diluted sample with the 490 µL of diluent in the second tube. This provides a 1,00-fold dilution of the sample.
Do not test plasma samples at dilutions less than 100-fold.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25℃. Performance of the assay at lower temperatures will result in lower absorbance values.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4℃ for future use.
2. Dispense 100 µL of HRP-conjugate into the wells.
3. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
4. Incubate on an orbital micro-plate shaker at 150 rpm and 25℃ for one hour.
5. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µL/well).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 µL of TMB into each well.
8. Incubate on an orbital micro-plate shaker at 150 rpm at 25℃ for 20 minutes.
9. After 20 minutes, stop the reaction by adding 100 µL of Stop solution to each well.
10. Gently mix. It is important to make sure that all the blue color changes to yellow.
11. Read absorbance at 450 nm with a plate reader within 5 minutes.

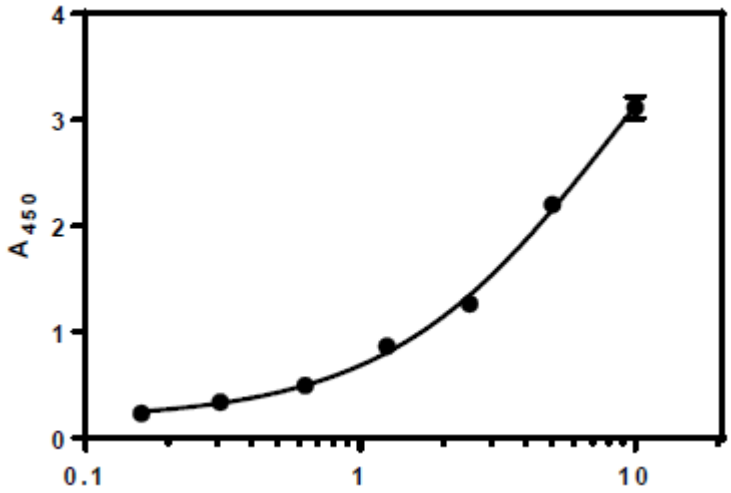
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus $\log_{10}$ of the concentration.
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the calibration curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and calibration curve in each experiment.

Ex-FABP (ng/mL)	Absorbance (450 nm)
10	3.119
5	2.201
2.5	1.264
1.25	0.865
0.625	0.494
0.313	0.339
0.156	0.231



Ex-FABP (ng/mL)

STORAGE

The unused kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

FOR RESEARCH USE ONLY

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