CHICKEN Ex-FABP ELISA
Life Diagnostics, Inc., Catalog Number: EXFABP-5

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 (ref 1). In studies at Life Diagnostics we found plasma levels ranging from 0.5 to >10 μg/ml.

PRINCIPLE OF THE ASSAY

The assay uses two chicken Ex-FABP antibodies developed at Life Diagnostics; one for solid-phase immobilization (microtiter wells) and one, conjugated to horseradish peroxidase (HRP), for detection. Standards 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. Absorbance is measured at 450 nm. The concentration of Ex-FABP is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Ex-FABP antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Ex-FABP stock (lyophilized)
- 20x Wash solution: TBS50-20, 50 ml
- Diluent: YD50-1, 2 x 50 ml
- TMB: TMB11-1, 11 ml
- Stop solution: SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized 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

STORAGE

The unused kit should be stored at 4°C 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.

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°C. Performance of the assay at lower temperatures will result in lower absorbance values.

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 deionized water.

STANDARD 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 standard remains stable for at least 1 day at room temperature but should be frozen at -20°C 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 standard 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 standard by mixing 250 μl of the 10 ng/ml standard with 250 μl of diluent in the tube labeled 5 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

To obtain values within the range of the standard curve, we suggest that serum or plasma be diluted at least 1000-fold (optimal dilution factors should be determined by the end user). A 1000-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 1000-fold dilution of the sample.

Do not test plasma samples at dilutions less than 100-fold.

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°C for future use.
2. Dispense 100 μl of HRP-conjugate into the wells.
3. Dispense 100 μl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
4. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C 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°C 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.

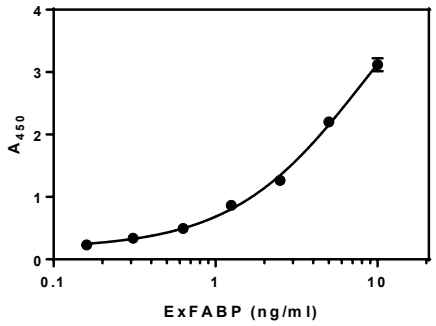
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus $\log_{10}$ of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the standard 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 plasma sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

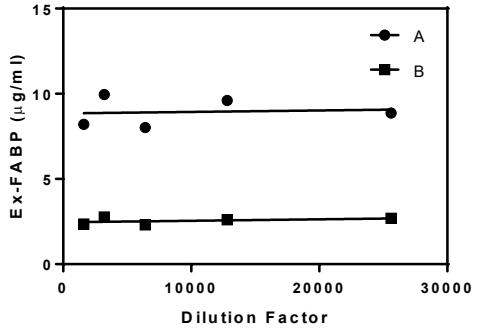
A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

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



ASSAY PERFORMANCE

To assess the linearity of the assay, two plasma samples containing naturally elevated Ex-FABP at concentrations of 8.93 µg/ml (A) and 2.55 µg/ml (B) were serially diluted with diluent YD50-1 to produce values within the dynamic range of the assay.



REFERENCES

1. Horvatic A. et.al. Quantitative proteomics using tandem mass tags in relation to the acute phase protein response in chicken challenged with Escherichia coli lipopolysaccharide endotoxin. J. Proteomics. 192:64-77 (2019)

Rev 10192020

For technical assistance please email us at
techsupport@lifediagnostics.com