RAT LIVER FATTY ACID BINDING PROTEIN (L-FABP) ELISA
Life Diagnostics, Inc., Catalog Number: LFABP-2

Rat L-FABP ELISA

INTRODUCTION

Liver fatty acid-binding protein (L-FABP) has a molecular weight of ~14 kDa and constitutes 2-5% of liver cytosolic protein.[1] Immunologically distinct intestinal and cardiac FABP isoforms also exist. All isoforms serve a role in fatty acid transport and metabolism. L-FABP is expressed primarily in the liver, but relatively high levels are found in intestinal tissue and it is expressed in other tissues also.[2] L-FABP has recently been identified as a possible biomarker for lung, kidney and liver disease.[3-5] The L-FABP kit manufactured by Life Diagnostics, Inc. does not recognize rat cardiac FABP. Cross-reactivity with intestinal FABP has not been investigated.

PRINCIPLE OF THE ASSAY

The rat L-FABP test kit is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-rat L-FABP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-rat L-FABP antibodies for detection. The test sample is diluted and incubated with conjugate in the microtiter wells for 60 minutes. This results in L-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies, and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of L-FABP is proportional to the optical density of the test sample, and the actual value is derived by reference to a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-Rat L-FABP Antibody Coated Microtiter Plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference Standard (lyophilized) containing 2 εg/ml rat L-FABP when reconstituted as detailed on the vial label
- Diluent, 25 ml
- 20x Wash Solution, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips.
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader at 450 nm wavelength with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Graph paper (PC graphing software is optional)

STORAGE

The kit should be stored at 2-8°C, and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

All reagents should be allowed to reach room temperature (18- 25^{o}C) before use.

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 rat L-FABP standard is provided in lyophilized form. Reconstitute as detailed on the vial label ***(the reconstituted standard should be aliquoted and frozen at -20^{o}C* after reconstitution if further use is intended)**.
2. Label 8 polypropylene or glass tubes as 1000, 500, 250, 125, 62.5, 31.25, 15.63 and 0 ng/ml.
3. Into the tube labeled 1000ng/mL, pipette the volume of diluent detailed on the L-FABP standard label. Then add the indicated volume of L-FABP stock (shown on the vial label) and mix gently. This provides the working 1000 ng/ml L-FABP standard.
4. Dispense 250 εl of diluent into the tubes labeled 500, 250, 125, 62.5, 31.25, 15.63 and 0 ng/ml
5. Prepare a 500 ng/ml standard by diluting and mixing 250 εl of the 1000 ng/ml standard with 250 εl of diluent in the tube labeled 500 ng/ml. Similarly prepare the 250, 125, 62.5, 31.25 and 15.63 ng/ml standards by serial dilution.

SAMPLE PREPARATION

Serum samples may be tested undiluted or after dilution with diluent.[a] The optimal dilution factor should be determined empirically.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and samples into the wells (we recommend that samples be tested in duplicate).
3. Add 100 μl of enzyme conjugate reagent into each well.
4. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 60 minutes.
5. Wash and empty the microtiter wells 5 times with 1x wash solution. Optimal results are obtained if a plate washer (400 μl/well) is used. However, if a plate washer is not available a squirt bottle may be used to manually wash the wells. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.

[a] If samples are tested at low dilutions (i.e., undiluted or 1:1 with diluent), we recommend that all samples be tested at the same dilution. This avoids minor artifacts occasionally caused by matrix differences between the diluent and serum samples.

7. Dispense 100 µl of TMB Reagent into each well.
8. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
9. 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 the optical density at 450 nm with a microtiter plate reader within 5 minutes.

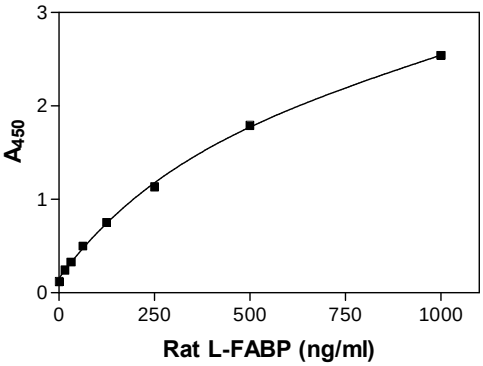
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of L-FABP in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of L-FABP in the serum/plasma sample.
5. If available, PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside the standard curve samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against L-FABP concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.

L-FABP (ng/ml)	Absorbance (450 nm)
1000	2.541
500	1.791
250	1.135
125	0.756
62.5	0.5015
31.25	0.330
15.63	0.246
0	0.122



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

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4. A Kamijo-Ikemori, et al. Urinary fatty acid binding protein in renal disease. Clin Chim Acta. 374:1-7 (2006)
5. M Afari Gyamfi, et al. The pathogenesis of ethanol versus methionine and choline deficient diet-induced liver injury. Biochem Pharm. 75:981-995 (2008)

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