KAMIYA BIOMEDICAL COMPANY

Rat Liver Fatty Acid Binding Protein ELISA

For the quantitative determination of Liver Fatty Acid Binding Protein (L-FABP) in rat serum.

Cat. No. KT-433

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY®*** Rat Liver Fatty Acid Binding Protein (L-FABP) ELISA is an enzyme immunoassay for the quantitative determination of Liver Fatty Acid Binding Protein (L-FABP) in rat serum. For research use only.

INTRODUCTION
Liver Fatty Acid Binding Protein (L-FABP) has a molecular weight of ~ 14 kDa and constitutes 2-5% of liver cytosolic protein. 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. L-FABP has recently been identified as a possible biomarker for lung, kidney and liver disease. The L-FABP kit manufactured by Kamiya Biomedical Company does not recognize rat cardiac FABP. Cross-reactivity with intestinal FABP has not been investigated.

PRINCIPLE
The ***K-ASSAY®*** Rat L-FABP ELISA 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 calibration curve.

COMPONENTS
- Anti-rat L-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 mL
- Rat L-FABP Calibrator (lyophilized), containing 2 µg/mL rat L-FABP when reconstituted as detailed on the vial label
- Diluent, 25 mL
- Wash Solution (20X), 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 de-ionized 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)

GENERAL INSTRUCTIONS
All reagents should be allowed to reach room temperature (18-25℃) 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 de-ionized water.

CALIBRATOR PREPARATION

1. The Rat L-FABP calibrator is provided in lyophilized form. Add the volume of diluent indicated on the vial label and mix gently until dissolved to obtain a 2 µg/mL (**the reconstituted calibrator should be aliquoted and frozen at -20°C after reconstitution if further use is intended).**
2. Label 8 polypropylene or glass tubes as 1,000, 500, 250, 125, 62.5, 31.25, 15.63 and 0 ng/mL.
3. Dispense 250 µL of diluent into the tube labeled 1,000 ng/mL and 250 µL of diluent into the remaining tubes.
4. Pipette 250 µL of the 2 µg/mL L-FABP calibrator into the tube labeled 1,000 ng/mL and mix. This provides the working 1,000 ng/mL L-FABP calibrator.
5. Prepare a 500 ng/mL calibrator by diluting and mixing 250 µL of the 1,000 ng/mL calibrator with 250 µL of diluent in the tube labeled 500 ng/mL. Similarly prepare the 250, 125, 62.5, 31.25 and 15.63 ng/mL calibrators by serial dilution.

SAMPLE PREPARATION

Serum samples may be tested undiluted or after dilution with diluent. 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 calibrators and diluted 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 adsorbent paper or paper towels to remove all residual droplets.
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

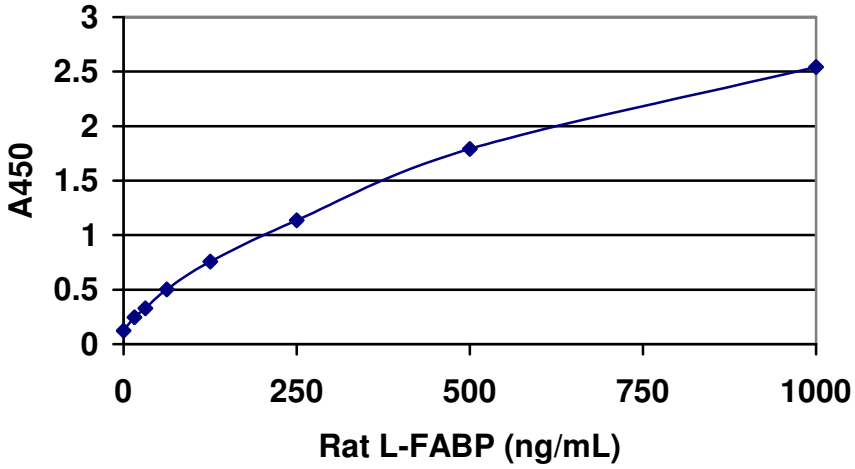
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators, and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentration 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 calibration 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 of the calibration curve samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density reading at 450 nm on the Y axis against L-FABP concentration 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)
1,000	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

Typical Rat L-FABP Calibration Curve



STORAGE

The unused kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date provided that the components are stored as described above.

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 reading.

FOR RESEARCH USE ONLY

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