KAMIYA BIOMEDICAL COMPANY

Rat H-FABP ELISA

For the quantitative determination of cardiac fatty acid binding protein (H-FABP) in rat serum or plasma.

Cat. No. KT-479

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** Rat H-FABP ELISA is an enzyme immunoassay for the quantitative determination of H-FABP in rat serum or plasma. For research use only.

INTRODUCTION
Fatty acid-binding proteins are cytoplasmic proteins of about 15 kDa that bind long chain fatty acids and play an important role in fatty acid metabolism. Different types of FABP have been detected including Heart FABP (H-FABP), liver FABP and intestinal FABP. Human cardiac muscle has a high content of H-FABP (10-20 mol % of cytoplasmic proteins) and H-FABP is a sensitive biomarker of myocardial necrosis that can be used to confirm or exclude a diagnosis of acute myocardial infarction (AMI). In AMI, H-FABP is rapidly released from damaged cardiomyocytes into the circulation due to its solubility and small size. Human clinical studies indicate that H-FABP levels are significantly increased above threshold within 3 hours of AMI and subsequently return to normal values in 12 to 24 hours. H-FABP has also been identified as a potential serum biomarker for stroke that is superior to either neuron specific enolase or S100B. Our Rat H-FABP ELISA is offered as a tool for investigation of heart damage in rat models of cardiovascular disease.

PRINCIPLE
The **K-*ASSAY*®** Rat H-FABP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses an affinity purified anti-rat H-FABP antibody for solid phase (microtiter wells) immobilization and a horseradish peroxidase (HRP) conjugated anti-rat H-FABP antibody for detection. The test sample is diluted and incubated with conjugate in the microtiter wells for 60 minutes. This results in rat H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes at room temperature, resulting 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 H-FABP is proportional to the optical density of the test sample.

COMPONENTS
- Anti-rat H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- HRP Conjugate Reagent, 11 mL
- Calibrator (lyophilized)
- 10X 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 water
- Polypropylene microcentrifuge tubes (1.5 mL)
- Vortex mixer or equivalent
- Absorbent paper or paper towel
- Micro-Plate shaker with mixing speed of ~100 rpm
- A microtiter plate reader capable of measuring absorbance at 450 nm, with 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°C) before use.
It may be necessary to dilute serum samples with the assay diluent in order to obtain values within the calibration range.

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.

DILUENT PREPARATION

The diluent is provided as a 10X stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10X stock with nine (9) volumes of distilled or de-ionized water.

CALIBRATOR PREPARATION

1. The rat H-FABP calibrator is provided as a lyophilized stock. Reconstitute with the volume of diluent indicated on the vial label. Mix gently until the contents of the vial dissolve.
2. Prepare a working 5 ng/mL calibrator according to the instructions on the calibrator vial label.
3. Label 7 polypropylene or glass tubes as 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0 ng/mL and pipette 250 µL of diluent into each tube.
4. Into the tube labeled 2.5 ng/mL, pipette and mix 250 µL of the 5 ng/mL calibrator. This provides the 2.5 ng/mL calibrator.
5. Similarly prepare the 1.25, 0.625, 0.313, 0.156 and 0.078 ng/mL calibrators by serial dilution.

Please Note: The reconstituted calibrator should be aliquoted and frozen at or below -20°C after reconstitution if future use is intended.

SAMPLE PREPARATION

Serum or EDTA plasma may be used in the assay. Avoid use of heparin plasma. Baseline levels of rat H-FABP are in the range of 1-2 ng/mL and can increase to 30 ng/mL or higher following cardiac injury. We recommend that samples be diluted 5-fold prior to assay. This may be achieved by mixing 50 µL of each test sample with 200 µL of 1x diluent.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators 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. This may be performed using either a plate washer (400 µL/well) or with a squirt bottle. 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 H-FABP in ng/mL from the calibration curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of H-FABP in the sample.
5. PC graphing software may be used for the above steps.

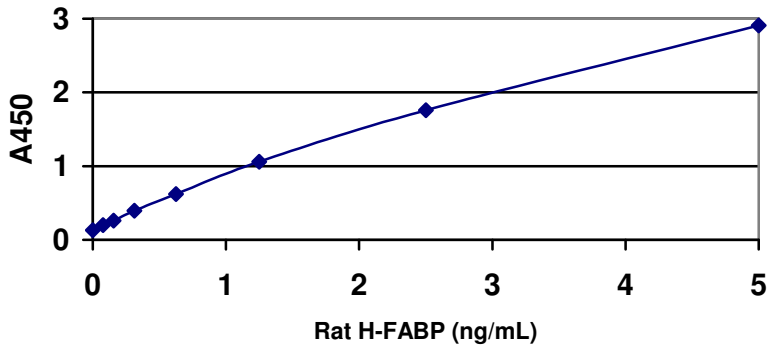
6. If the OD_{450} values of samples fall outside of the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density reading at 450 nm on the Y axis against H-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. Each user should obtain his or her data and calibration curve in each experiment.

H-FABP (ng/mL)	Absorbance (450 nm)
5	2.909
2.5	1.758
1.25	1.059
0.625	0.622
0.313	0.397
0.156	0.261
0.078	0.198
0	0.129

Typical Rat H-FABP Calibration Curve



STORAGE

The calibrator stock provided with the kit should be frozen at or below -20℃ on receipt. The remainder of the 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 package insert 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.

FOR RESEARCH USE ONLY

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