KAMIYA BIOMEDICAL COMPANY

Rabbit H-FABP ELISA

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

Cat. No. KT-476

For Research Use Only.

PRODUCT INFORMATION

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

INTRODUCTION
Fatty acid-binding proteins (FABP's) are a class of 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 high content of 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) and for monitoring recurrent infarction in humans. 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 elevated 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 Rabbit H-FABP ELISA is offered as a tool for investigation of heart damage in rabbit models of cardiovascular disease.

PRINCIPLE
The **K-*ASSAY*®** Rabbit H-FABP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes an affinity purified anti-rabbit H-FABP antibody for solid phase (microtiter wells) immobilization and a different anti-rabbit H-FABP antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in H-FABP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60-minute incubation at room temperature on an orbital shaker, the wells are washed with wash buffer to remove unbound-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-rabbit H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 13 mL
- Calibrator (250 µL), 1,000 ng/mL rabbit H-FABP
- 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 0-2 OD or greater and a

bandwidth of 10 nm or less
- 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. The dilution factor must be determined empirically and we recommend that all samples be similarly diluted.

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. Thaw the 1,000 ng/mL H-FABP calibrator.
2. Label 7 polypropylene microcentrifuge tubes as 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/mL.
3. Dispense 540 µL of diluent into the tube labeled 100 ng/mL and 300 µL of diluent into the remaining tubes.
4. Pipette 60 µL of the 1,000 ng/mL H-FABP calibrator into the tube labeled 100 ng/mL and mix. This provides the working 100 ng/mL H-FABP calibrator.
5. Prepare a 50 ng/mL calibrator by diluting and mixing 300 µL of the 100 ng/mL calibrator with 300 µL of diluent in the tube labeled 50 ng/mL. Similarly prepare the 25, 12.5, 6.25, 3.12 and 1.56 ng/mL calibrators by serial dilution.
6. Return the 1,000 ng/mL H-FABP calibrator to the -20°C freezer if future use is intended.

SAMPLE PREPARATION

Serum should be prepared from a whole blood specimen obtained by approved techniques. Plasma may be used also.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and samples into the appropriate wells.
3. Add 100 µL of enzyme conjugate reagent into each well.
4. Incubate on an orbital micro-plate shaker at 100 rpm at room temperature (18-25°C) for 60 minutes.
5. Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container or using a plate washer.
6. Wash the microtiter wells 5 times with wash solution. Preferably, a plate washer should be used with a wash of 5 x 400 µL.
7. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
8. Dispense 100 µL of TMB Reagent into each well. Gently mix for 10 seconds.
9. Incubate at room temperature in the dark for 20 minutes.
10. Stop the reaction by adding 100 µL of Stop Solution to each well.
11. Gently mix for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*
12. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes. In the event that the high calibrator OD readings exceed the range of the spectrophotometer, absorbance values for all wells may be determined at 405 nm instead.

CALCULATION OF RESULTS

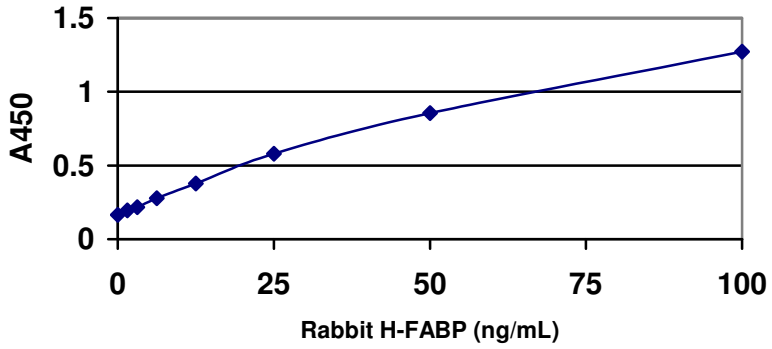
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators, and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical 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. If available, PC graphing software may be used.

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)
100	1.273
50	0.856
25	0.579
12.5	0.378
6.25	0.278
3.125	0.216
1.56	0.196
0	0.165

Typical Rabbit 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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