KAMIYA BIOMEDICAL COMPANY

Monkey H-FABP ELISA

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

Cat. No. KT-550

For Research Use Only.

PRODUCT INFORMATION

Monkey H-FABP ELISA
Cat. No. KT-550

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

INTRODUCTION
Fatty acid-binding proteins (FABP) are cytosolic proteins of about 15 kDa. They bind long chain fatty acids and play an important role in fatty acid metabolism. Heart, liver and intestinal FABP isoforms exist. Heart has a high content of FABP (10-20 mol % of cytoplasmic proteins) and heart FABP (H-FABP) has proved to be a sensitive biomarker of myocardial necrosis in humans. H-FABP is rapidly released into the circulation from damaged cardiac muscle. In humans serum levels increase significantly within 1-4 hours of muscle injury and return to normal within 12 to 24 hours. Because H-FABP is also expressed in skeletal muscle, it is necessary to exclude or control for skeletal muscle inquiry before ascribing H-FABP elevations to cardiac injury. However, in the absence of cardiac injury H-FABP is a useful biomarker of skeletal muscle injury. Validation studies at KAMIYA BIOMEDICAL COMPANY revealed basal monkey H-FABP levels of ~ 20 ng/mL with levels in excess of 400 ng/mL in animals with muscle injury.

PRINCIPLE
The **K-ASSAY®** Monkey H-FABP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses a mouse monoclonal anti-H-FABP antibody for solid phase (microtiter wells) immobilization and a different horseradish peroxidase (HRP) conjugated mouse monoclonal anti-H-FABP antibody for detection. Calibrators and diluted samples are incubated with the HRP conjugate in the microtiter wells for 60 minutes. This results in H-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 H-FABP is proportional to optical density. H-FABP concentrations are determined by reference to a calibration curve.

COMPONENTS
- Anti-H-FABP antibody coated 96 well plate (12 strips of 8 wells)
- Enzyme Conjugate Reagent, 11 mL
- Reference calibrator (lyophilized)
- Diluent, 50 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 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

Please read and understand the instructions thoroughly before using the kit.
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 reference calibrator is provided in lyophilized form. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved **(the reconstituted calibrator should be aliquoted and frozen at or below -20℃ if further use is intended).**
2. Label 8 polypropylene or glass tubes as 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39 and 0 ng/mL.
3. Into the tube labeled 25 ng/mL, pipette 479.2 µL of diluent. Then add 20.8 µL of reconstituted calibrator and mix gently. This provides the 25 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, and 0 ng/mL.
5. Pipette 250 µL of the 25 ng/mL H-FABP calibrator into the tube labeled 12.5 ng/mL and mix. This provides the working 12.5 ng/mL H-FABP calibrator. Similarly prepare the 6.25, 3.13, 1.56, 0.78, and 0.39 ng/mL calibrators by serial dilution.

SAMPLE PREPARATION

We suggest that serum samples initially be tested after a 5-fold dilution with the diluent provided with the kit.

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 calibrators and 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℃) for 60 minutes.
5. Aspirate the contents of the microtiter wells and wash the 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℃) 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

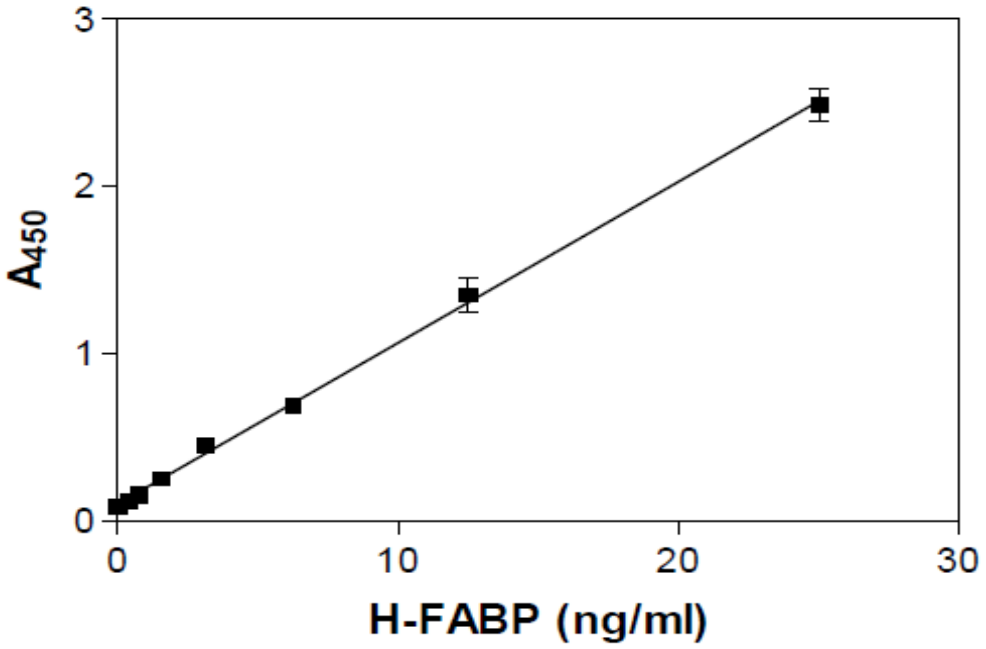
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical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

H-FABP (ng/mL)	Absorbance (450 nm)
25	2.499
12.5	1.436
6.25	0.686
3.13	0.479
1.56	0.240
0.78	0.154
0.39	0.11
0	0.082



STORAGE

The lyophilized reference calibrator must be stored 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.
3. All steps should be completed as quickly as accuracy allows.
4. This kit is intended for use with serum, not plasma.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com