KAMIYA BIOMEDICAL COMPANY

Pig H-FABP ELISA

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

Cat. No. KT-473

For Research Use Only.

PRODUCT INFORMATION

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

INTRODUCTION
Fatty acid-binding proteins (FABP) are cytosolic proteins of about 15 kD. 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. Serum/plasma levels are significantly increased within 1-4 hours of muscle injury and values 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 injury before ascribing H-FABP elevations to cardiac injury.

PRINCIPLE
The **K-*ASSAY*®** Pig H-FABP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-pig H-FABP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-pig H-FABP antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 30 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 the optical density of the test sample.

COMPONENTS
- Anti-pig H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 mL
- Calibrator (lyophilized)
- 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 de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with mixing speed of ~150 rpm
- A microtiter plate reader capable of measuring absorbance at 450 nm, 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 Pig H-FABP calibrator is provided in lyophilized form. Reconstitute the lyophilized Pig H-FABP reference calibrator to a concentration of 500 ng/mL by adding the volume of de-ionized or distilled water indicated on the vial label. Mix gently until dissolved.
2. Label 7 polypropylene or glass tubes as 25, 12.5, 6.25, 3.13, 1.56, 0.787 and 0 ng/mL.
3. Dispense 570 µL of diluent into the tube labeled 25 ng/mL and 300 µL of diluent into the remaining tubes.
4. Pipette 30 µL of the 500 ng/mL H-FABP calibrator into the tube labeled 25 ng/mL and mix. This provides the working 25 ng/mL H-FABP calibrator.
5. Prepare a 12.5 ng/mL calibrator by diluting and mixing 300 µL of the 25 ng/mL calibrator with 300 µL of diluent in the tube labeled 12.5 ng/mL. Similarly prepare the 6.25, 3.13, 1.56 and 0.78 ng/mL calibrators by serial dilution.

Please Note: The reconstituted calibrator is stable for 1 week at 4℃, but should be aliquoted and frozen at -20℃ after reconstitution if future use is intended.

SAMPLE PREPARATION

1. Pig serum or plasma samples may need to be diluted prior to assay in order to obtain values within the range of the calibration curve. The dilution factor must be determined empirically. We suggest that the researcher chooses a sample likely to have the highest H-FABP level and run an initial test with that sample to determine an optimum dilution factor. Other samples should subsequently be tested at that dilution.
2. Dilution should be performed with the yellow diluent provided with the kit.
3. If samples are to be tested in duplicate a final volume of 250 µL of diluted sample is sufficient.

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. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25℃) for 45 minutes.
4. Remove the incubation mixture either with a plate washer or by flicking plate contents into a waste container.
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. Add 100 µL of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25℃) for 30 minutes.
9. Wash as detailed in 4 to 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 µL of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25℃) for 20 minutes.
13. Stop the reaction by adding 100 µL of Stop Solution to each well.
14. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
15. 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

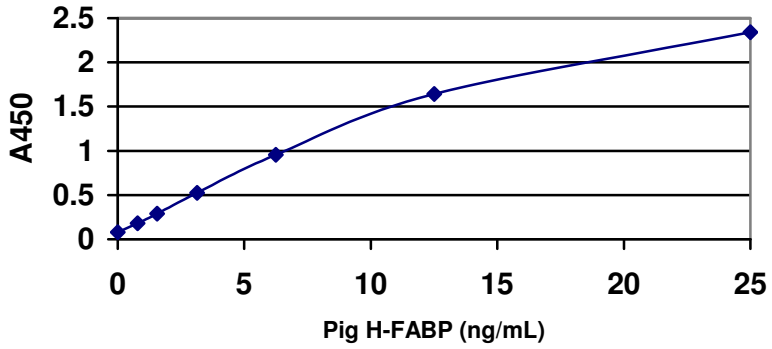
serum/plasma sample.
5. If available, PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside of the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density reading at 450 nm on the Y axis against 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)
25	2.341
12.5	1.643
6.25	0.954
3.13	0.523
1.56	0.289
0.78	0.180
0	0.079

Typical Pig H-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 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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