KAMIYA BIOMEDICAL COMPANY

Dog H-FABP ELISA

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

Cat. No. KT-463

For Research Use Only.

PRODUCT INFORMATION

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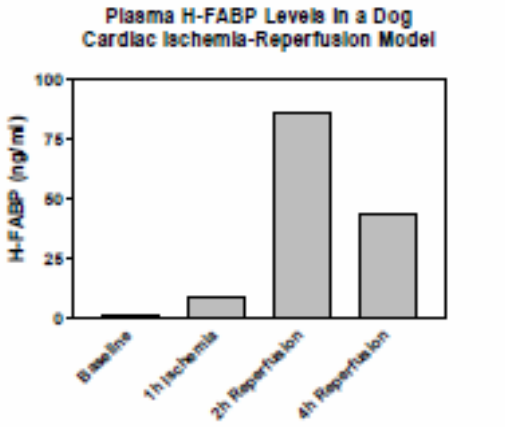
PRODUCT
The **K-ASSAY®** Dog H-FABP ELISA is an enzyme immunoassay for the quantitative determination of H-FABP in dog serum or plasma. For research use only.

NTRODUCTION
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 inquiry before ascribing H-FABP elevations to cardiac injury. As shown in the figure below, H-FABP serves as a useful marker of cardiac injury in dogs.



PRINCIPLE
The **K-ASSAY®** Dog H-FABP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-dog H-FABP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-dog 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-dog H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 mL
- Calibrator (lyophilized), containing 60 ng/mL dog H-FABP
- 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 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°C) 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.

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 dog H-FABP calibrator is provided in lyophilized form. Reconstitute the lyophilized dog H-FABP reference calibrator to a concentration of 60 ng/mL by adding the volume of de-ionized or distilled water indicated on the vial label. Mix gently until dissolved.
2. Label 8 polypropylene or glass tubes as 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0 ng/mL.
3. Dispense 400 µL of diluent into the tube labeled 20 ng/mL and 300 µL of diluent into the remaining tubes.
4. Pipette 200 µL of the 60 ng/mL H-FABP calibrator into the tube labeled 20 ng/mL and mix. This provides the working 20 ng/mL H-FABP calibrator.
5. Prepare a 10 ng/mL calibrator by diluting and mixing 300 µL of the 20 ng/mL calibrator with 300 µL of diluent in the tube labeled 10 ng/mL. Similarly prepare the 5, 12.5, 2.5, 1.25, 0.625 ng/mL calibrators by serial dilution.

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

SAMPLE PREPARATION

General Note: In plasma samples from a dog ischemia-reperfusion model we found that peak H-FABP levels of ~85 ng/mL were achieved 2h after reperfusion. Baseline levels were approximately 1 ng/mL. We suggest that samples initially be tested after a 5-fold dilution in 1x sample diluent.

1. Dispense 240 µL of 1x diluent into separate tubes.
2. Pipette and mix 60 µL of each serum/plasma sample into a tube containing 240 µL of diluent. This provides a 5 fold diluted sample.

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°C) for 45 minutes.
4. Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1X wash solution. This may be performed using either a plate

washer (350 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°C) 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°C) 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 15 minutes.

CALCULATION OF RESULTS

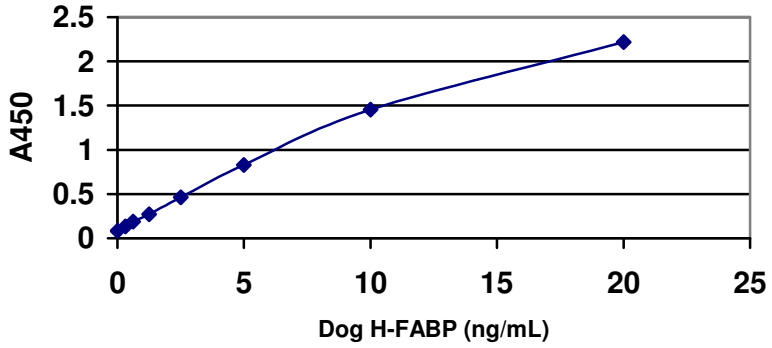
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators, and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical 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)
20	2.218
10	1.455
5	0.830
2.5	0.464
1.25	0.272
0.625	0.187
0.3125	0.135
0	0.084

Typical Dog 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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