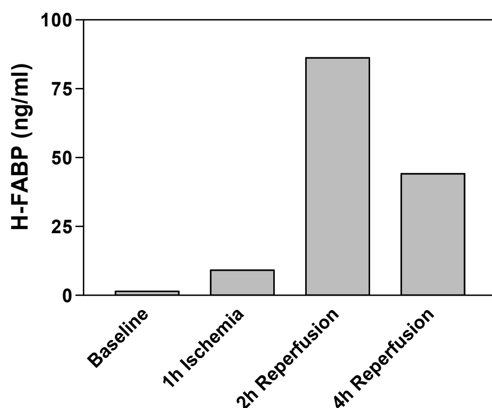
DOG CARDIAC FATTY ACID BINDING PROTEIN ELISA KIT
Life Diagnostics, Inc., Catalog Number: 2310-4

Enzyme Immunoassay for the Quantitative Determination of Dog Cardiac Fatty Acid Binding Protein (H-FABP)

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. As shown in the Figure below, H-FABP serves as a useful marker of cardiac injury in dogs.

Plasma H-FABP Levels in a Dog Cardiac Ischemia-Reperfusion Model



PRINCIPLE OF THE TEST

The 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.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti- dog H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard (lyophilized), containing 60 ng/ml dog H-FABP when reconstituted as detailed on the vial label
- 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 deionized 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)

STORAGE OF TEST KIT

The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

All reagents should be allowed to reach room temperature (18- 25°C) before use.

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 deionized water.

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 deionized water.

STANDARD PREPARATION

1. The dog H-FABP standard is provided in lyophilized form. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved to obtain a 60 ng/ml stock ***(the reconstituted standard should be aliquoted and frozen at -20°C after reconstitution if further use is intended)***.
2. Label 8 polypropylene or glass tubes as 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0 ng/ml

Life Diagnostics, Inc., P.O. Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

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 standard into the tube labeled 20 ng/ml and mix. This provides the working 20 ng/ml H-FABP standard.
5. Prepare a 10 ng/ml standard by diluting and mixing 300 µl of the 20 ng/ml standard 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 standards by serial dilution.

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 standards 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 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 absorbent 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 and 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

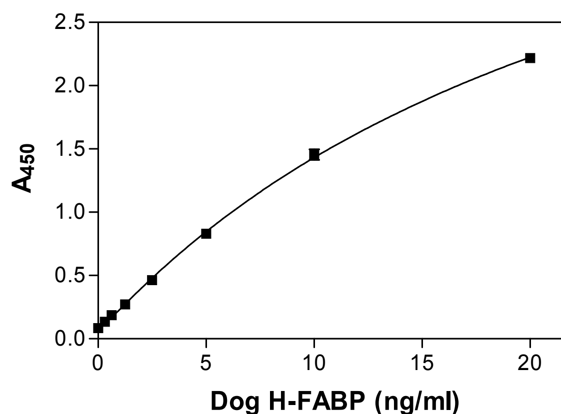
1. Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or 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 standard curve.
4. Multiply the derived concentrations 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 the standard curve samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against H-FABP concentrations 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)
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

Representative Dog H-FABP Standard Curve



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 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.