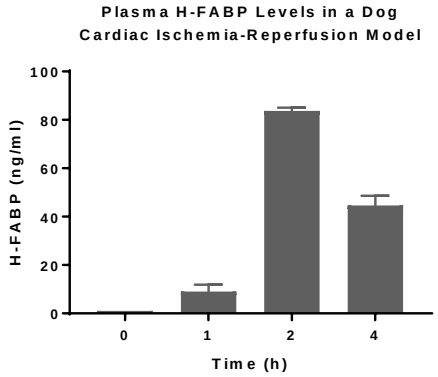
DOG H-FABP ELISA
Life Diagnostics, Inc., Cat. No. HFABP-4

INTRODUCTION

Fatty acid-binding proteins are ≈15 kDa cytoplasmic proteins involved in fatty acid transport and metabolism. The isoform expressed in heart (H-FABP) is highly abundant, representing 10-20 mol % of total cytoplasmic protein. H-FABP is released into blood after cardiac injury and provides a sensitive biomarker of myocardial necrosis in several species.[1-5] H-FABP is expressed in both cardiac and skeletal muscle. When used as a cardiac biomarker, it is therefore important to rule out skeletal muscle injury. In the absence of cardiac injury, H-FABP can also be used as a biomarker of skeletal muscle damage. As shown in the figure below, we found that H-FABP levels peaked two hours after cardiac ischemia-reperfusion in dogs.



PRINCIPLE OF THE ASSAY

The ELISA uses two H-FABP antibodies. One is used for solid phase immobilization (microtiter wells). The other is conjugated to horse radish peroxidase (HRP) and used for detection. Standards and diluted samples are incubated in the microtiter wells with HRP conjugate for one hour. This results in H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP conjugate. TMB is added and incubated for 20 minutes. If H-FABP is present a blue color develops. Color development is stopped by addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of H-FABP is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- *Anti-H-FABP coated plate (12 x 8-well strips)*
- *H-FABP Stock, lyophilized*
- *HRP Conjugate, 11 ml*
- *Diluent: CSDT50-1, 50 ml*
- *20x Wash solution: TBS50-20, 50 ml*
- *TMB: TMB11-1, 11 ml*
- *Stop solution: SS11-1, 11 ml*

Materials required but not provided:

- *Pipettors and tips*
- *Distilled or deionized water*
- *Polypropylene or glass tubes*
- *Vortex mixer*
- *Absorbent paper or paper towels*
- *Plate incubator/shaker*
- *Plate washer*
- *Plate reader capable of measuring absorbance at 450 nm.*
- *Curve fitting software*

STORAGE CONDITIONS

Store the lyophilized stock at or below -20°C. The remainder of the kit should be stored at 2°C to 8°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. *All reagents should be allowed to reach room temperature before use.*
2. *Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.*
3. *The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.*
4. *Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.*

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. *Reconstitute the lyophilized H-FABP stock with de-ionized or distilled water as detailed on the vial label. Mix gently until dissolved.*
2. *Label 7 polypropylene tubes as* 100, 33.3, 11.1, 3.7, 1.23, 0.41 and 0.14 *ng/ml.*
3. *In the tube labeled 100 ng/ml, pipette the volume of 1x diluent detailed on the stock vial label. Then add the indicated volume of stock and mix gently. This provides the 100 ng/ml standard.*
4. *Pipette 400* µl *of diluent into the tubes labeled* 33.3, 11.1, 3.7, 1.23, 0.41 and 0.14 *ng/ml.*
5. *Prepare a 33.3 ng/ml standard by diluting and mixing 200* µl *of the 100 ng/ml standard with 400* µl *of diluent in the tube labeled 33.3 ng/ml. Similarly prepare the remaining standards by three-fold serial dilution.*

The reconstituted H-FABP stock should be frozen at or below -20°C if future use is intended.

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

SAMPLE COLLECTION AND PREPARATION

Serum, EDTA-plasma, or urine may be used. Do not use heparin-plasma. Baseline levels of H-FABP are ≈1 ng/ml in dogs. After cardiac injury, we found levels as high as levels 80 ng/ml. We recommend that serum and plasma samples be diluted at least 5-fold prior to assay. Urine samples should be diluted at least 20-fold to avoid matrix effects. Optimal dilutions must be determined empirically.

ASSAY PROCEDURE

1. *Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 2°C to 8°C for future use.*
2. *Dispense 100 µl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).*
3. *Add 100 µl of HRP-conjugate into each well.*
4. *Incubate on a plate shaker at 150 rpm and 25°C for one hour.*
5. *Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).*
6. *Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.*
7. *Dispense 100 µl of TMB into each well.*
8. *Incubate on a plate shaker at 150 rpm and 25°C for 20 minutes.*
9. *After 20-minutes, 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 absorbance at 450 nm with a plate reader within 5 minutes.*

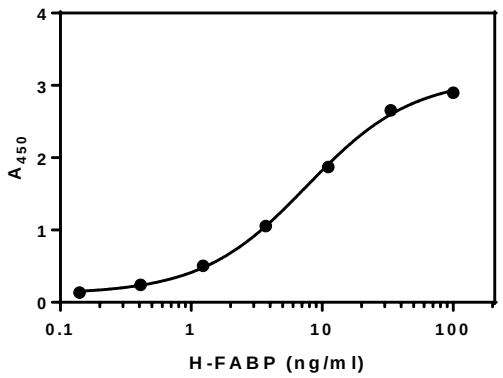
CALCULATION OF RESULTS

1. *Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus log_{10} of the concentration.*
2. *Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = log_{10} concentration) and determine the concentration of the samples.*
3. *Multiply the derived concentration by the dilution factor to determine the actual concentration in the original sample.*
4. *If the A_{450} values fall outside the standard curve, samples should be diluted appropriately and re-tested.*

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This is for illustration only. A standard curve must be generated for each experiment.

H-FABP (ng/ml)	A_{450}
100	2.899
33.3	2.656
11.1	1.870
3.7	1.054
1.23	0.504
0.41	0.242
0.14	0.134



REFERENCES

1. *Moayeri M. et al. The heart is an early target of anthrax lethal toxin in mice: A protective role for neuronal nitric oxide synthase (nNOS). PLoS Pathog 5(5): e1000456. doi:10.1371/journal.ppat.1000456*
2. *Ziegler, M. et al. Highly sensitive detection of minimal cardiac ischemia using positron emission tomography imaging of activated platelets. Sci. Rep. 6, 38161; doi: 10.1038/srep38161 (2016)*
3. *Yoshitani K. et al. Strain-specific differences in sensitivity to myocardial injury after cardioplegic arrest in the rat. 107: A1326 (2007)*
4. *Hasic S. et al. Heart-type fatty-acid binding protein and its relation with morphological changes in rat myocardial damage model induced by isoproterenol. Bosn J Basic Med Sci. 11(4): 240-244 (2011)*
5. *Uitterdijk A. et al. Serial measurement of hFABP and high-sensitivity troponin-I post-PCI in STEMI: how fast and accurate can myocardial infarct size and n-reflow be predicted? Am J Physiol Heart Circ Physiol. 305:H1104–H1110 (2013)*

Rev 06162020

For technical assistance please email us at techsupport@lifediagnostics.com