RABBIT H-FABP ELISA
Life Diagnostics, Inc., Cat. No. HFABP-10

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 using it 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.

PRINCIPLE OF THE ASSAY

The ELISA uses two different 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, 2 vials, lyophilized
- HRP Conjugate, 11 ml
- Diluent; YD25-1, 25 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 2-8°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. The rabbit H-FABP standard is provided as a lyophilized stock. Reconstitute with the volume of diluent indicated on the vial label. Mix gently until the contents of the vial dissolve. This provides the working 100 ng/ml standard. ***The reconstituted standard should be aliquoted and frozen at or below -20°C after reconstitution if additional use is intended***.
2. Label six microcentrifuge tubes as 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml. Pipette 250 µl of diluent into each tube.
3. Pipette and mix 250 µl of the 100 ng/ml standard with 250 µl of diluent in the tube labeled 50 ng/ml. This provides the 50 ng/ml standard.
4. Similarly prepare the remaining standards by two-fold serial dilution.

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

SAMPLE COLLECTION AND PREPARATION

Serum should be used in the assay. We found levels of ~2 ng/ml H-FABP in normal rabbit serum. In a study of ischemia reperfusion injury, levels increased to ~90 ng/ml one hour after reperfusion. It may be necessary to dilute samples with the assay diluent to obtain samples within range of the standard curve. If so, dilute samples with the diluent provided. 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 2-8°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.

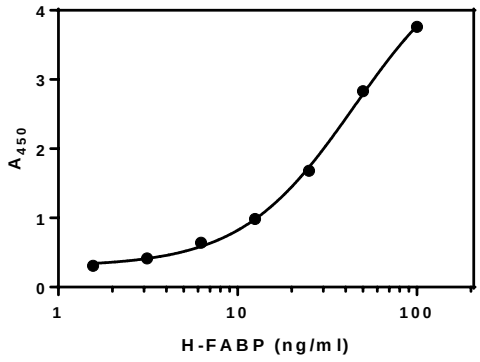
CALCULATION OF RESULTS

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	3.762
50	2.831
25	1.681
12.5	0.986
6.25	0.641
3.13	0.415
1.56	0.307



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Rev 010518

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