RABBIT CARDIAC FATTY ACID-BINDING PROTEIN (H-FABP) ELISA
Life Diagnostics, Inc., Catalog Number: HFABP-10

Rabbit Cardiac Fatty Acid-Binding Protein (H-FABP) ELISA

INTRODUCTION

Fatty acid-binding proteins (FABPs) are a class of cytoplasmic proteins of about 15 kDa that bind and transport long chain fatty acids. Different isoforms of FABP have been identified including heart FABP (H-FABP), liver FABP and intestinal FABP. Cardiac muscle has high content of H-FABP (10-20 mol % of cytoplasmic proteins), and H-FABP is a sensitive biomarker of myocardial injury. Following cardiac damage, H-FABP is rapidly released from damaged cardiomyocytes into circulation due to its solubility and small size. Studies in rabbits have demonstrated that H-FABP levels peak as early as one hour after cardiac damage. Our rabbit H-FABP kit is offered as a tool for investigation of heart damage in rabbit models of cardiovascular disease.

PRINCIPLE OF THE TEST

The H-FABP test kit is a solid phase enzyme-linked immunosorbent assay (ELISA). The assays uses an affinity purified anti-rabbit H-FABP antibody for solid phase (microtiter wells) immobilization and a horseradish peroxidase (HRP) conjugated anti-rabbit H-FABP antibody for detection. The test sample is diluted and incubated with conjugate in the microtiter wells for 60 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. A solution of TMB Reagent is added and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, changing the color to yellow, and the sample is measured spectrophotometrically at 450 nm. The concentration of H-FABP is proportional to the optical density.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-rabbit H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- HRP Conjugate Reagent, 11 ml
- Reference standard stock (lyophilized), 2 vials
- Diluent, 25 ml
- 20x Wash Buffer, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Deionized water
- Polypropylene microcentrifuge tubes (1.5 ml)
- Vortex mixer or equivalent
- Absorbent paper or paper towels
- Plate shaker with an approximate mixing speed of 100 rpm
- Microtiter plate reader (450 nm wavelength) with an optical density range of 0-4 OD
- Graph paper (PC graphing software is optional)

STORAGE

The reference standard stocks provided with the kit should be stored at or below -20°C on receipt. **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 to minimize exposure to damp air. Test kits will remain stable until the expiration date shown on the kit package, provided that the components are stored as described above.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. It may be necessary to dilute serum samples with the assay diluent in order to obtain values within the standard range.

WASH BUFFER PREPARATION

The wash buffer 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 7 polypropylene or glass tubes as 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/ml, and pipette 250 µl of diluent into each tube.
3. Into the tube labeled 50 ng/ml, pipette and mix 250 µl of the 100 ng/ml standard. This provides the 50 ng/ml standard.
4. Similarly prepare the 25, 12.5, 6.25, 3.125, and 1.56 ng/ml standards by serial dilution.

SAMPLE PREPARATION

Serum should be used in the assay. We found baseline levels of rabbit H-FABP to be approximately 2 ng/ml. 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 in order to obtain samples within range of the standard curve. If so, dilute samples with the diluent provided.

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. Add 100 µl of enzyme conjugate reagent into each well.
4. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 60 minutes.
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 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. Dispense 100 µl of TMB Reagent into each well.
8. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
9. 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 the optical density at 450 nm with a microtiter plate reader *within 5 minutes*. In the event that the high standard exceeds 4 OD units, either eliminate the 100 ng/ml standard from the analysis or measure optical density at 405 nm. Absorbance values at 405 nm will be lower, but the precision of the assay is not significantly diminished.

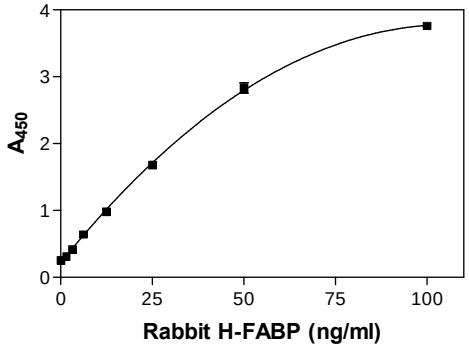
CALCULATION OF RESULTS

1. Calculate the average absorbance values 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 concentration by the dilution factor to determine the actual concentration of H-FABP in the sample.
5. PC graphing software may be used for the above steps.

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 standard curve is for the purpose of illustration only and should not be used to calculate unknowns.

H-FABP (ng/ml)	A_{450}
100	3.762
50	2.831
25	1.681
12.5	0.986
6.25	0.641
3.125	0.415
1.56	0.307
0	0.255



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.

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