RAT CARDIAC FATTY ACID-BINDING PROTEIN (H-FABP) ELISA TEST KIT
Life Diagnostics, Inc., Catalog Number: 2310-2

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

**Materials provided with the kit:**
- Anti-rat H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8).
- Enzyme Conjugate Reagent, 13 ml.
- Reference standard (100 µl), 1000 ng/ml rat H-FABP
- Store the standard at -20°C

**Materials required but not provided:**
- Diluent (25 ml)
- Wash Buffer (20x stock, 50 ml)
- TMB Reagent (One-Step) 11 ml.
- Stop Solution (1N HCl), 11 ml.

**Introduction**
Fatty acid-binding proteins (FABP's) are a class of cytoplasmic proteins of about 15 kDa that bind long chain fatty acids and play an important role in fatty acid metabolism. Different types of FABP have been detected including Heart FABP (H-FABP), Liver FABP and Intestinal FABP. Human cardiac muscle has high content of FABP (10-20 mol % of cytoplasmic proteins) and H-FABP is a sensitive biomarker of myocardial necrosis that can be used to confirm or exclude a diagnosis of acute myocardial infarction (AMI) and for monitoring recurrent infarction in humans. In AMI, H-FABP is rapidly released from damaged cardiomyocytes into the circulation due to its solubility and small size. Human clinical studies indicate that H-FABP levels are significantly elevated above threshold within 3 hours of AMI and subsequently return to normal values in 12 to 24 hours. H-FABP has also been identified as a potential serum biomarker for stroke that is superior to either neuron specific enolase or S100B.

Our rat H-FABP kit is offered as a tool for investigation of heart damage in rat models of cardiovascular disease.

**Principle of the test**
The H-FABP Quantitative test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes an affinity purified anti-rat H-FABP antibody for solid phase (microtiter wells) immobilization and a different anti-rat H-FABP antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in H-FABP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60-minute incubation at room temperature on an orbital shaker, the wells are washed with wash buffer to remove unbound-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. Thecolor development is stopped with the addition of Stop Solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of H-FABP is proportional to the color intensity of the test sample.

**Materials and components**

**Specimen Collection and Preparation**
Serum should be prepared from a whole blood specimen obtained by approved techniques. Plasma may be used also.

**Storage of Test Kit and Instrumentation**
The reference standard stock provided with the kit should be frozen 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 desiccants to minimize exposure to damp air. Test kits will remain stable until the expiration date shown, provided that the components are stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater.

**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.

**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. The dilution factor must be determined empirically and we recommend that all samples be similarly diluted.

**Standard Preparation**
1. Thaw the 1000 ng/ml H-FABP standard.
2. Label 7 polypropylene microcentrifuge tubes as 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0 ng/ml
3. Dispense 585 μl of diluent into the tube labeled 25 ng/ml and 300 μl diluent into the remaining tubes.
4. Pipette 15 μl of the 1000 ng/ml H-FABP standard into the tube labeled 25 ng/ml and mix. This provides the working 25 ng/ml H-FABP standard.
5. Prepare a 12.5 ng/ml standard by diluting and mixing 300 μl of the 25 ng/ml standard with 300 μl of diluent in the tube labeled 12.5 ng/ml. Similarly prepare the 6.25, 3.125, 1.56 and 0.78 ng/ml standards by serial dilution.
6. Return the 1000 ng/ml H-FABP standard to the -20°C freezer if future use is intended.

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and samples into appropriate wells.
3. Dispense 100 μl of Enzyme Conjugate Reagent into each well.
4. Incubate on an orbital micro-plate shaker at 100 rpm at room temperature (18-25°C) for 60 minutes.
5. Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container or using a plate washer.
6. Wash the microtiter wells 5 times with wash buffer. Preferably, a plate washer should be used with a wash of 5 x 400 μl.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
8. Dispense 100 μl TMB Reagent into each well. Gently mix for 10 seconds.
9. Incubate at room temperature in the dark for 20 minutes.
10. Stop the reaction by adding 100 μl of Stop Solution to each well.
11. Gently mix for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*
12. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes. *In the event that the high standard OD readings exceed the range of the spectrophotometer, absorbance values for all wells may be determined at 405 nm instead.*

**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. If available, PC graphing software may be used.

**TYPICAL STANDARD CURVE**

Results of a typical standard run with optical density readings at 450nm shown on the Y axis against H-FABP concentrations shown on the X axis. This standard 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 standard curve in each experiment.

<table>
<thead>
<tr>
<th>H-FABP (ng/ml)</th>
<th>Absorbance (450 nm)</th>
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<tbody>
<tr>
<td>0</td>
<td>0.132</td>
</tr>
<tr>
<td>0.78</td>
<td>0.278</td>
</tr>
<tr>
<td>1.56</td>
<td>0.363</td>
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<tr>
<td>3.125</td>
<td>0.571</td>
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<tr>
<td>6.25</td>
<td>0.858</td>
</tr>
<tr>
<td>12.5</td>
<td>1.267</td>
</tr>
<tr>
<td>25</td>
<td>1.652</td>
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</tbody>
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**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.

**REFERENCES**


