

HUMAN CARDIAC FATTY ACID-BINDING PROTEIN (H-FABP) ELISA TEST KIT

OxisResearch Catalog Number: 11230

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

*****FOR RESEARCH USE ONLY*****

Store at 2 to 8°C

INTENDED USE

For the quantitative determination of Human Cardiac Fatty Acid-Binding Protein (H-FABP) concentration in serum

INTRODUCTION

Fatty acid-binding proteins (FABP) are tissue specific intracellular molecules of about 15 kD. They are a class of cytoplasmic proteins that bind long chain fatty acid and play an important role in the intracellular utilization of fatty acids. Different types of FABP have been detected and these include Heart FABP, Liver FABP and Intestinal FABP, etc. Human cardiac muscle has high content of FABP (10-20 mol % of cytoplasmic proteins). Heart FABP (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 of a recurrent infarction³. In AMI, H-FABP is rapidly released from damaged cardiomyocytes into the circulation due to its small size. H-FABP levels are significantly elevated above their threshold level within 3 hours after AMI and subsequently return to normal values in 12 to 24 hours⁷. More recently, H-FABP has been identified as a potential serum biomarker for stroke that is superior to either neuron specific enolase or S100B⁸.

The normal levels of H-FABP range from 1.6 ng/ml to an upper level of 19 ng/ml in various studies of cardiovascular disease¹⁻⁷. H-FABP increases slightly with age.

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 goat anti-H-FABP antibody for solid phase (microtiter wells) immobilization and the same goat anti-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 60-minute incubation at room temperature on an orbital shaker, the wells are washed with distilled water 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. The color 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

Materials provided with the kit:

- Goat anti-H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8).
- Enzyme Conjugate Reagent, 13 ml.
- H-FABP reference standards, containing 0, 1, 2.5, 5, 10, 25 and 100 ng/ml, Lyophilized.
- TMB Reagent (One-Step) 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes and tips, 50 µl, 100 µl and 1.0 ml.
- Distilled water.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Micro-Plate incubator/shaker with an approximate mixing speed of 250 rpm.
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater.
- Graph paper (PC graphing software is optional).

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. The reconstituted FABP standards will be stable at 2 - 8 °C for 30 days. For long-term stability,

aliquot and store the reconstituted FABP standards at -20 °C or below.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standard, specimens, and controls (if appropriate) into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Incubate on an orbital micro-plate shaker at 250 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.
6. Rinse and flick the microtiter wells 5 times distilled or deionized water. **(Please do not use tap water.)**
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water 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.

CALCULATION OF RESULTS

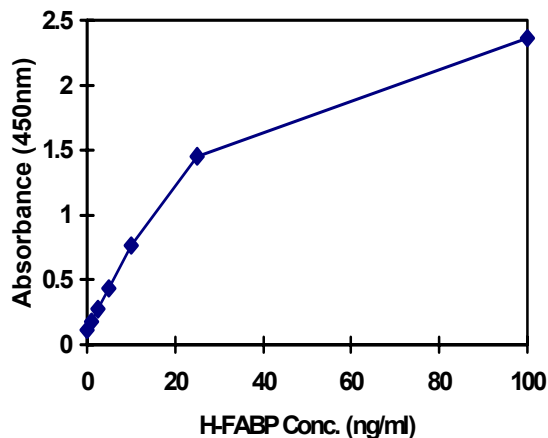
1. Calculate the average absorbance values (A_{450}) for each set of reference standards, control, and samples.
2. Constructed a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the 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

H-FABP (ng/ml)	Absorbance (450 nm)
0	0.111
1	0.181
2.5	0.275
5	0.437
10	0.768
25	1.455
100	2.364

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.



EXPECTED VALUES AND SENSITIVITY

Each laboratory must establish its own normal ranges based on patient population. The information provided below is cited from references 1 – 7:

Adult	ng/ml
Healthy individuals	1.6-19
After AMI	>19

LIMITATIONS OF THE PROCEDURE (FOR RESEARCH USE ONLY)

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.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

REFERENCES

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