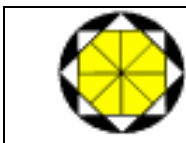


HUMAN CARDIAC SPECIFIC FATTY ACID-BINDING PROTEIN (H-FABP) ENZYME IMMUNOASSAY TEST KIT

Catalog Number: BC-1123



BIOCHECK, INC
323 Vintage Park Drive
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Enzyme Immunoassay for the Quantitative Determination of Human Cardiac Fatty Acid-Binding Protein (H-FABP) in Serum and Plasma

FOR RESEARCH USE ONLY

Store at 2 to 8°C

PROPRIETARY AND COMMON NAMES

BioCheck H-FABP Enzyme Immunoassay

INTENDED USE

For the quantitative determination of Human Cardiac Fatty Acid-Binding Protein (H-FABP) concentration in serum, citrate-plasma, EDTA-plasma, and heparin-plasma.

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 infarction¹. 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 hours². More recently, H-FABP has been identified as a potential serum biomarker for stroke that is superior to either neuron specific enolase or S100B³.

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 disease^{1,2,4-8}. 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)⁹⁻¹⁰. 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 sequentially with these antibodies, resulting in H-FABP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After two separate 60-minute incubation steps at room temperature on an orbital shaker, the wells are washed with Wash Buffer to remove unbound-labeled antibodies. TMB Reagent is added and incubated for 20 minutes at room temperature with shaking, resulting in the development of a blue color. The blue 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.
- Sample Diluent, 30 ml
- Enzyme Conjugate Reagent, 13 ml.
- H-FABP reference standards, containing 0, 1, 2.5, 5, 10, and 25 ng/ml, Lyophilized.
- 20X Wash Buffer
- 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 750 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

1. Serum Samples: Whole blood should be collected using standard venipuncture techniques. Let sample sit at room temperature for 2 hours before centrifuging at 2,500 rpm for 20 minutes at 4°C. Remove serum supernatant. Serum can be stored at 2 - 8°C for 5 hours. Store at -20°C or below, if not assayed immediately.
2. Plasma Samples: Whole blood should be collected using standard venipuncture techniques. Let sample sit at room temperature for 2 hours before centrifuging at 2,500 rpm for

20 minutes at 4°C. Remove plasma supernatant. Plasma can be stored at 2 - 8°C for 5 hours. Store at -20°C or below, if not assayed immediately.

3. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).

WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum and plasma samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists¹¹⁻¹².
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. This test kit is for research use only.

STORAGE CONDITIONS AND INSTRUMENTATION

1. Store the unopened test kits at 2-8°C upon receipt and when it is not in use, until the expiration date shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.
3. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.

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 about 20 minutes and mix gently. The reconstituted FABP standards will be stable at 2 - 8 °C for 48 hours. For long-term stability, aliquot and store the reconstituted FABP standards at -20 °C or below.
3. Working Wash Buffer: Preparation of 1X Wash Buffer from 20X Stock. Add 50 ml of 20X Wash Buffer Stock to 950 ml of DI H₂O. The Working Wash Buffer is stable at 2 - 8°C for 30 days.

SAMPLE PREPARATION

1. Serum and plasma samples should be diluted 20 fold prior to use. Prepare a series of small tubes (i.e., 1.5 ml

microcentrifuge tubes) and mix 15 ul of serum sample with 285 ul of Sample Diluent.

2. Samples with expected H-FABP concentrations over 500 ng/ml may be quantitated by further dilution (10 fold) of the 20-fold diluted solution with sample diluent (i.e. 30 ul of the 20-fold diluted sample with 270 ul of sample diluent).

INSTRUMENTATION

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.

PROCEDURAL NOTES

1. No more than 6 strips (48 wells) should be used for each assay. If more than 6 strips are used, time-effect may be observed.
2. Pipetting Recommendations (single and multi-channel): Pipetting of all standards, samples, and controls should be completed within 8 minutes.
3. Strips should be placed in the middle of the plate to eliminate edge effect.
4. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
5. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standard, diluted specimens, and diluted controls into appropriate wells.
3. Incubate on orbital micro-plate shaker at 750 rpm at room temperature (18-25°C) for 60 minutes.
4. Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container.
5. Rinse and flick the microtiter wells 5 times with 300 ul Working Wash Buffer.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
8. Incubate on orbital micro-plate shaker at 750 rpm at room temperature (18-25°C) for 60 minutes.
9. Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container.
10. Rinse and flick the microtiter wells 5 times with 300 ul Working Wash Buffer.
11. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
12. Dispense 100 µl TMB Reagent into each well. Gently mix for 10 seconds.
13. Incubate at on orbital micro-plate shaker in the dark for 20 minutes.

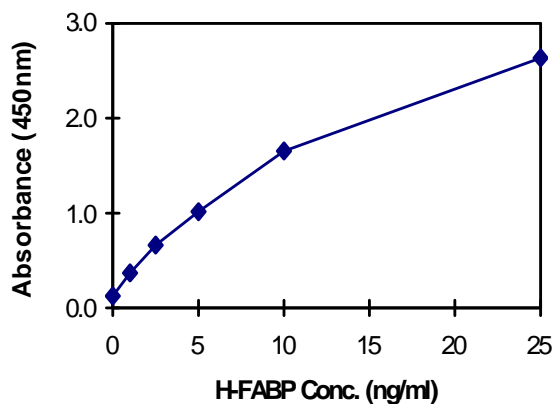
14. Stop the reaction by adding 100 μ l of Stop Solution to each well.
15. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
16. Read the optical density at 450 nm with a microtiter plate reader **within 15 minutes.**

EXAMPLE OF STANDARD CURVE

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

H-FABP (ng/ml)	A450
0	0.125
1.0	0.368
2.5	0.661
5	1.015
10	1.652
25	2.637

Representative Human Cardiac FABP Standard Curve



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. The corresponding concentration of H-FABP (ng/ml) can be determined from the standard curve using the mean absorbance value for each sample. Depending on the experience and/or the availability of the computer capability, other methods of data reduction may be employed.
4. The obtained values of serum or plasma patient samples should be multiplied by the dilution factor of 20 to obtain H-FABP results in ng/ml.
5. Samples with H-FABP values greater than 500 ng/ml should be further diluted 10-fold after the initial 20-fold dilution (total dilution 1:200), and the final H-FABP values should be multiplied by 200 to obtain H-FABP results in ng/ml.
6. Samples with H-FABP values less than 5 ng/ml after multiplied by the dilution factor should be reported as less than 5 ng/ml.

EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population. The information provided below is cited from references 1, 2, 4-8:

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

CORRELATION OF H-FABP TO NORMAL AND ABNORMAL PATIENT SAMPLES

A. High Myoglobin Patient Serum Samples:

Sample	Myoglobin (ng/ml)	H-FABP (ng/ml)
1	346	200
2	669	127
3	732	28
4	1,499	294
5	5,074	775
6	13,943	375
7	14,061	380

B. Likely AMI Patient Serum Samples:

Sample	Troponin I (ng/ml)	CK-MB (ng/ml)	H-FABP (ng/ml)
8	0.5	6	165
9	0.7	1.6	37
10	3.6	9.3	40
11	5	17	14
12	7	4	46
13	10.2	28.3	15
14	30	15	37

C. High CRP Patient Serum Samples:

Sample	CRP (mg/L)	H-FABP (ng/ml)
15	14	12
16	18	12
17	65	52
18	86	12
19	86	26
20	102	26
21	135	18

D. Apparently Normal Patient Samples:

Sample	MPO (ng/ml)	CRP (mg/L)	H-FABP (ng/ml)	H-FABP (ng/ml)	H-FABP (ng/ml)	H-FABP (ng/ml)
Sample Type	Serum	Serum	Serum	Citrate Plasma	EDTA Plasma	Heparin Plasma
22	63	0.5	5.4	5.1	5.0	5.9
23	102	1.5	10	8.2	13.3	12.1
24	114	1.7	7.5	8.5	8.9	10.9
25	156	0.2	5.9	6.5	7.0	8.6
26	176	0.7	8.8	6.8	8.6	9.5
27	162	0.9	6.8	7.3	9.3	10.9
28	224	0.2	<5	<5	<5	5

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.
3. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

PERFORMANCE CHARACTERISTICS

1. Minimum Detectable Sensitivity

The minimum detectable concentration of H-FABP ELISA assay as measured by 2SD from the mean of a zero standard is estimated to be 0.25 ng/ml.

Serum or plasma samples have a minimum detectability of 5 ng/ml after multiplying by the dilution factor of 1:20.

2. Precision

a. Intra-Assay Precision

Within-run precision was determined by 20 or 14 replicate determinations of three different serum samples in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3
# Replicates	20	20	14
Mean H-FABP (ng/ml)	19	104	866
S.D.	1.31	8.37	68.88
C.V. (%)	7.0	8.0	8.0

b. Inter-Assay Precision

Between-run precision was determined by 5 replicate measurements of four different serum samples over a series of 5 individually calibrated assays. Between-assay variability is shown below:

Serum Sample	1	2	3	4
# Replicates	25	25	25	25
Mean H-FABP (ng/ml)	24	86	180	369
S.D.	2.99	9.44	16.73	39.80
C.V. (%)	12.6	10.9	9.3	10.8

3. Whole Plate Uniformity

Three samples, one diluted 1:20, one diluted 1:40, and one diluted 1:80 were pipetted into 48 wells place in the center of the plate. The time to load the plate from the first well to the last

Sample	Ave. A ₄₅₀	Std. Dev.	%CV
1 (1:20)	0.349	0.015	4.3
2 (1:40)	1.634	0.125	7.6
3 (1:80)	1.635	0.091	5.6

8 minutes.

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PERFORMANCE CHARACTERISTICS

4. Linearity and Recovery Studies

a. Linearity

4 patient serum samples were serially diluted to determine linearity. The mean recovery was 127.5%.

#	Dilution	Expected Conc. (ng/ml)	Observed Conc. (ng/ml)	% Expected
1	1:20	215	215	--
	1:40		252	117.2%
	1:80		360	167.4%
	1:160		414	192.6%
Mean = 159.1%				
2	1:20	246	246	--
	1:40		195	79.3%
	1:80		241	98.0%
	1:160		276	112.2%
Mean = 96.5%				
3	1:20	128	128	--
	1:40		134	104.7%
	1:80		161	125.8%
	1:160		190	148.4%
Mean = 126.3%				
4	1:20	52	52	--
	1:40		54	103.8%
	1:80		68	130.8%
	1:160		78	150.0%
Mean = 128.2%				

b. Recovery

Various patient serum samples of known FABP levels were combined and assayed in duplicate. The mean recovery was 100.4%.

PAIR NUMBER	EXPECTED [H-FABP] (ng/ml)	OBSERVED [H-FABP] (ng/ml)	% RECOVERY
1	487.3	370.3	76.0%
2	111.3	120.3	108.1%
3	114.7	115.3	100.5%
4	72.3	60.4	83.3%
5	29.9	30.2	101.0%
6	12.4	16.6	133.1%

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