



hFABP ELISA

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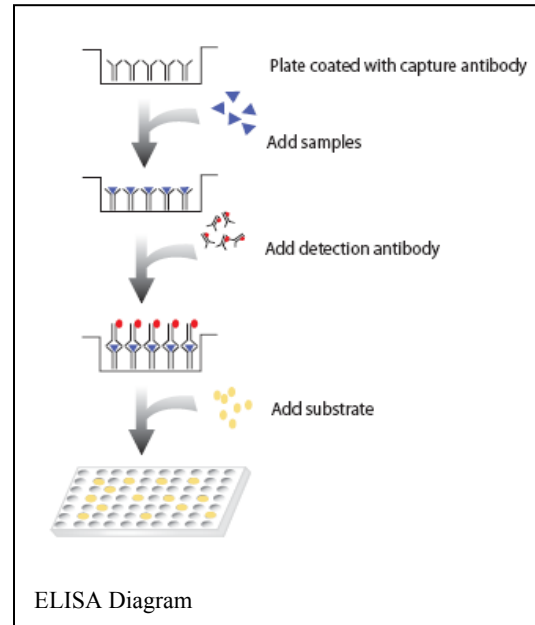
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Introduction

Heart fatty acid binding protein (hFABP) is a novel small cytosolic protein that is abundant in the heart. It is highly cardiac-specific (i.e. expressed primarily in cardiac tissue), but is also expressed at low concentrations in tissues outside the heart. Heart 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 infarction (1). After myocardial ischemic damage, hFABP level is significantly elevated above its threshold level within 3 hours after AMI and subsequently returns to normal values in 12 to 24 hours (2). In AMI, hFABP is rapidly released from damaged cardiomyocytes into the circulation due to its small size. More recently, hFABP has been identified as a potential serum biomarker for stroke that is superior to either neuron specific enolase or S100B (3). The normal levels of hFABP range from 1.6ng/ml to an upper level of 19 ng/ml in various studies of cardiovascular disease (1,2,4,8). Heart FABP increases slightly with age.

Principle of the assay

The hFABP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes an affinity purified goat anti-hFABP antibody for immobilization on the microtiter wells and the same antibody conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with these antibodies, resulting in hFABP being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. An HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of hFABP is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Materials provided with the kit

- Antibody-coated microtiter plate with 96 wells.
- Sample Diluent, 30 ml
- H-FABP reference standards, containing 0, 1, 2.5, 5, 10, and 25 ng/ml, Lyophilized.
- 20X Wash Buffer
- Enzyme Conjugate Reagent, 13 ml.
- TMB Reagent (One-Step) 11 ml.
- Stop Solution (1N HCl), 11 ml.

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Warning 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 samples, should be considered potentially infectious. It is recommended that the reagents and patient samples be handled according to the OSHA Standard on Bloodborne Pathogens (5) or other appropriate national biohazard safety guidelines or regulations (6-8).
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.

Reagent preparation

All reagents should be allowed to reach room temperature (18-25°C) before use.

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 standards will be stable for up to 8 hours when stored sealed at 2-8°C.

Discard the reconstituted Standards after 8 hours. To assure maximum stability of the reconstituted Standards, they should be aliquoted and frozen (-20°C or below) immediately after reconstitution has been achieved. Each aliquoted Standard should be frozen and thawed only once.

Assay procedure

1. Add 100µl of blocking solution to each well and incubate for 1 hour at room temperature with gentle shaking.
2. Aspirate each well.
3. Add 50 µl of Standard, control, or sample per well and incubate for 1.5 hours at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200µl of Wash buffer. Repeat the process three times for a total of four washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 100µl of anti- hFABP HRP conjugate to each well and incubate for 1.5 hours at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 200µl of fresh prepared mix of substrate A and B at equal volume to each well.
8. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
9. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

References

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Example of standard curve

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

