

PIG H-FABP ELISA

Life Diagnostics, Inc., Cat. No. HFABP-9

INTRODUCTION

Fatty acid-binding proteins are approximately 15 kDa cytoplasmic proteins involved in fatty acid transport and metabolism. The isoform expressed in heart (H-FABP) is highly abundant, representing 10-20 mol % of total cytoplasmic protein. H-FABP is released into blood after cardiac injury and provides a sensitive biomarker of myocardial necrosis in several species.¹⁻⁵ H-FABP is expressed in both cardiac and skeletal muscle. When using it as a cardiac biomarker, it is therefore important to rule out skeletal muscle injury. In the absence of cardiac injury, H-FABP can also be used as a biomarker of skeletal muscle damage.

PRINCIPLE OF THE ASSAY

The assay uses affinity purified H-FABP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated rabbit H-FABP antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 30 minutes. This results in H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If H-FABP is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of H-FABP is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-H-FABP coated plate (12 x 8-well strips)
- H-FABP Stock, lyophilized
- HRP Conjugate, 11 ml
- Diluent: YD25-1, 25 ml
- 20x Wash solution: TBS50-20, 50 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE CONDITIONS

Store the lyophilized stock at or below -20°C. The remainder of the kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

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.

STANDARD PREPARATION

1. Reconstitute the lyophilized H-FABP stock with de-ionized or distilled water as detailed on the vial label. Mix gently until dissolved.
2. Label 7 polypropylene tubes as 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 ng/ml.
3. In the tube labeled 25 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of stock and mix gently. This provides the 25 ng/ml standard.
4. Pipette 250 μ l of diluent into the tubes labeled 12.5, 6.25, 3.13, 1.56, 0.781 and 0.39 ng/ml.
5. Prepare a 12.5 ng/ml standard by diluting and mixing 250 μ l of the 25 ng/ml standard with 250 μ l of diluent in the tube labeled 12.5 ng/ml. Similarly prepare the remaining standards by two-fold serial dilution.

The reconstituted H-FABP stock should be frozen at or below -20°C if future use is intended.

SAMPLE COLLECTION AND PREPARATION

Serum, EDTA-plasma, or urine may be used. Do not use heparin-plasma. Baseline levels of H-FABP are in the range of 1 – 2 ng/ml. After cardiac injury, levels can exceed 30 ng/ml. We recommend that serum and plasma samples be diluted 5-fold prior to assay. Urine samples should be diluted at least 20-fold to avoid matrix effects.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 μ l of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 μ l/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 μ l of HRP-conjugate into each well.

7. Incubate on a plate shaker at 150 rpm and 25°C for 30 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 μ l of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 μ l of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

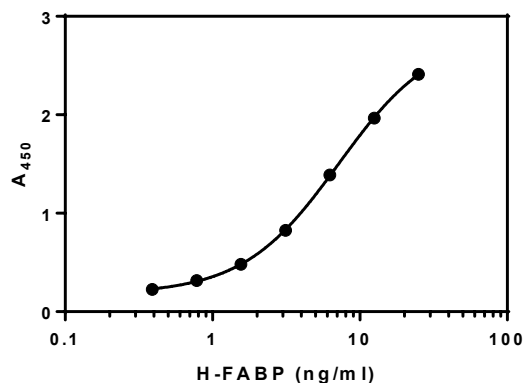
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus \log_{10} of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = \log_{10} concentration) and determine the concentration of the samples.
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the original sample.
4. If the A_{450} values fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This is for illustration only. A standard curve must be generated for each experiment.

H-FABP (ng/ml)	A_{450}
25	2.410
12.5	1.967
6.25	1.389
3.13	0.827
1.56	0.481
0.78	0.316
0.39	0.227



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Rev 03032021

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