RAT H-FABP ELISA
Life Diagnostics, Inc., Catalog Number: HFABP-1

Rat Cardiac Fatty Acid Binding Protein (H-FABP) ELISA

INTRODUCTION

Fatty acid-binding proteins are 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 a high content of H-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). In AMI, H-FABP is rapidly released from damaged cardiomyocytes into 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 \neuron specific enolase and S100B. Our high sensitivity rat H-FABP kit is offered as a tool for investigation of heart damage in rat models of cardiovascular disease.[1]

PRINCIPLE OF THE TEST

The H-FABP quantitative test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assays uses an affinity purified anti-rat H-FABP antibody for solid phase (microtiter wells) immobilization and a horseradish peroxidase (HRP) conjugated anti-rat H-FABP antibody for detection. The test sample is diluted and incubated with conjugate in the microtiter wells for 60 minutes. This results in rat H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-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, changing the color to yellow, and the test sample is measured spectrophotometrically at 450 nm. The concentration of H-FABP is proportional to the optical density.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-rat H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- HRP Conjugate Reagent, 11 ml.
- Reference standard stock (lyophilized)
- 10x Diluent, 25 ml
- 20x Wash Buffer, 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene microcentrifuge tubes (1.5 ml)
- Vortex mixer or equivalent
- Absorbent paper or paper towels
- Plate shaker with an approximate mixing speed of 100 rpm
- Microtiter plate reader (450 nm wavelength) with an optical density range of 0-4 OD
- Graph paper (PC graphing software is optional)

STORAGE

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 desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date shown on the kit package, provided that the components are stored as described above.

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.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use, estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

WASH BUFFER PREPARATION

The wash buffer 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. The rat H-FABP standard is provided as a lyophilized stock. Reconstitute with the volume of diluent indicated on the vial label. Mix gently until the contents of the vial dissolve. ***The reconstituted standard should be aliquoted and frozen at or below -20°C after reconstitution if additional use is intended***.
2. Prepare a working 5 ng/ml standard according to the instructions on the reference standard vial label.
3. Label 7 polypropylene or glass tubes as 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0 ng/ml, and pipette 250 µl of diluent into each tube.
4. Into the tube labeled 2.5 ng/ml, pipette and mix 250 µl of the 5ng/ml standard. This provides the 2.5 ng/ml standard.
5. Similarly prepare the 1.25, 0.625, 0.313, 0.156, 0.078 ng/ml standards by serial dilution.

SAMPLE PREPARATION

Serum, EDTA plasma or urine may be used in the assay. Avoid use of heparin plasma. Baseline levels of H-FABP are in the range of 1 – 2 ng/ml and can increase to 30 ng/ml or higher in rats following cardiac injury.[1,2] We recommend that serum and plasma samples be diluted 5-fold prior to assay. This may be achieved by mixing 50 µl of each test sample with 200 µl of 1x diluent. Urine

samples should be diluted at least 20-fold in order to avoid matrix effects.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and samples into the wells (we recommend that samples be tested in duplicate).
3. Add 100 µl of enzyme conjugate reagent into each well.
4. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 60 minutes.
5. Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (400 µl/well) or a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 µl of TMB Reagent into each well.
8. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
9. Stop the reaction by adding 100 µl of Stop Solution to each well.
10. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
11. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.

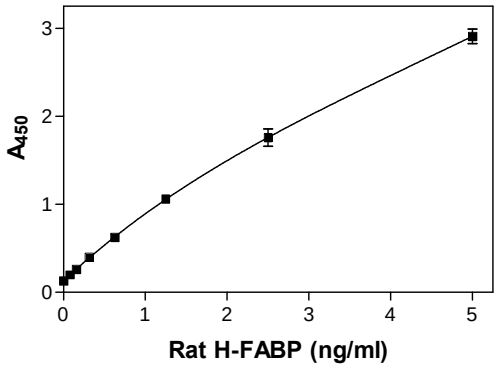
CALCULATION OF RESULTS

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. Multiply the derived concentration by the dilution factor to determine the actual concentration of H-FABP in the sample.
5. PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y-axis against H-FABP concentrations on the X-axis is shown below. This standard curve is for the purpose of illustration only and should not be used to calculate unknowns.

H-FABP (ng/ml)	Absorbance (450 nm)
5	2.909
2.5	1.758
1.25	1.059
0.625	0.622
0.313	0.397
0.156	0.261
0.078	0.198
0	0.129



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

1. Yoshitani K. et. al.. Strain-Specific Differences in Sensitivity to Myocardial Injury after Cardioplegic Arrest in the Rat. Am. Soc. Anesthesiol., Annual Meeting Abstracts, Oct. 15 2007, A1326
2. Makhsous M. et.al. Elevation in the serum and urine concentration of injury-related molecules after the formation of deep tissue injury in a rat spinal cord injury pressure ulcer model. PM&R 2(11):1063-1065 (2010)

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For technical assistance please email us at
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