

# MONKEY CARDIAC FATTY ACID BINDING PROTEIN ELISA KIT

Life Diagnostics, Inc., Catalog Number: 2310-7

## ELISA for the Quantitative Determination of Cardiac Fatty Acid Binding Protein (H-FABP) in Monkey Serum

### INTRODUCTION

Fatty acid-binding proteins (FABP) are cytosolic proteins of about 15 kDa. They bind long chain fatty acids and play an important role in fatty acid metabolism. Heart, liver, and intestinal FABP isoforms exist. Heart has a high content of FABP (10-20 mol % of cytoplasmic proteins), and heart FABP (H-FABP) has proved to be a sensitive biomarker of myocardial necrosis in humans. H-FABP is rapidly released into the circulation from damaged cardiac muscle. In humans serum levels increase significantly within 1-4 hours of muscle injury and return to normal within 12 to 24 hours. Because H-FABP is also expressed in skeletal muscle, it is necessary to exclude or control for skeletal muscle injury before ascribing H-FABP elevations to cardiac injury. However, in the absence of cardiac injury H-FABP is a useful biomarker of skeletal muscle injury. Validation studies at Life Diagnostics Inc., revealed basal monkey H-FABP levels of ~ 20 ng/ml with levels in excess of 400 ng/ml in animals with muscle injury.

### PRINCIPLE OF THE TEST

The monkey H-FABP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses a mouse monoclonal anti-H-FABP antibody for solid phase (microtiter wells) immobilization and a different horseradish peroxidase (HRP) conjugated mouse monoclonal anti-H-FABP antibody for detection. Standards and diluted samples are incubated with the HRP conjugate in the microtiter wells for 60 minutes. This results in H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies, and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of H-FABP is proportional to optical density. H-FABP concentrations are determined by reference to a standard curve.

### MATERIALS AND COMPONENTS

#### **Materials provided with the kit:**

- Anti-H-FABP antibody coated 96 well plate (12 strips of 8 wells)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard<sup>1</sup> (lyophilized)
- Diluent, 50 ml
- 20x Wash Solution, 50 ml

<sup>1</sup>Due to international import/export restrictions of monkey derived products the reference standard provided with this kit is of non monkey origin. The standard curve obtained with this material is identical to that obtained with native monkey H-FABP.

- 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 or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Graph paper (PC graphing software is optional)

### STORAGE OF TEST KIT

The lyophilized reference standard must be stored 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 for six months from the date of purchase provided that the components are stored as described above.

### GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (18- 25°C) before use.

### 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. The reference standard is provided in lyophilized form. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved (**the reconstituted standard should be aliquoted and frozen at or below -20°C if further use is intended**).
2. Label 8 polypropylene or glass tubes as 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, and 0 ng/ml.
3. Into the tube labeled 250 ng/ml, pipette the volume of diluent detailed on the reference standard vial label. Then add the indicated volume of reconstituted standard and mix gently. This provides the 250 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 125, 62.5, 31.3, 15.6, 7.8, 3.9, and 0 ng/ml.
5. Pipette 250 µl of the 250 ng/ml H-FABP standard into the tube labeled 125 ng/ml H-FABP standard. This provides the working 125 ng/ml H-FABP standard. Similarly prepare the 62.5, 31.3, 15.6, 7.8, and 3.9 ng/ml standards by serial dilution.

### SAMPLE PREPARATION

**We suggest that samples initially be tested after a 5-fold dilution with the diluent provided with the kit.**

1. Dispense 240 µl of 1x diluent into separate tubes.

- Pipette and mix 60  $\mu\text{l}$  of each serum sample into a tube containing 240  $\mu\text{l}$  of diluent. This provides a 5-fold diluted sample.

### ASSAY PROCEDURE

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

### CALCULATION OF RESULTS

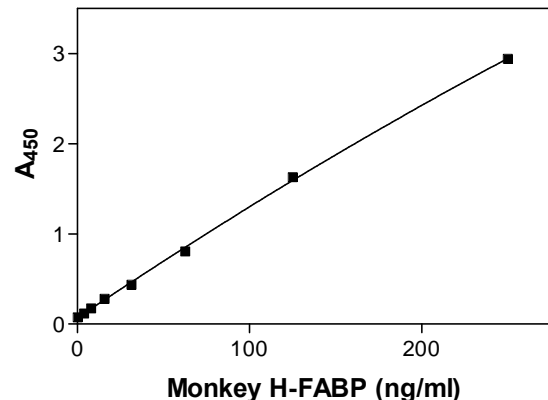
- Calculate the average absorbance values ( $A_{450}$ ) for each set of reference standards and samples.
- Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
- Using the mean absorbance value for each sample, determine the corresponding concentration of H-FABP in ng/ml from the standard curve.
- Multiply the derived concentrations by the dilution factor to determine the actual concentration of H-FABP in the serum sample.
- If available, PC graphing software may be used for the above steps.
- If the  $A_{450}$  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 450 nm on the Y axis against H-FABP concentration on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.

H-FABP (ng/ml)	Absorbance (450 nm)
250.0	2.94
125.0	1.631
62.5	0.806
31.3	0.439
15.6	0.28
7.8	0.176
3.9	0.118
0.0	0.075

### Typical Monkey H-FABP Standard Curve



### LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- All steps should be completed as quickly as accuracy allows.
- This kit is intended for use with serum, not plasma.