Human L-FABP

EIA Package Insert

DPGLFABP

FOR RESEARCH USE ONLY

Not for use in Diagnostic Procedures

## Table of Contents:

**ASSAY DESCRIPTION**

- Intended Use
- Background
- Summary and Explanation of the Test
- Materials Provided for 96 Determinations
- Storage and Shelf Life After First Opening
- Materials Required but not Provided

**ASSAY PROTOCOL**

- Warnings and Precautions
- Sample Preparation
- Component Preparation
- Assay Procedure
- Procedure Summary Schematic
- Performance
- Calculation of Analytical Results

**TECHNICAL SUPPORT**

## ASSAY DESCRIPTION

## INTENDED USE

The Diapharma human Liver-Fatty Acid Binding Protein (L-FABP or FABP1) ELISA kit is to be used for the *in vitro* quantitative determination of human L-FABP in serum, plasma, urine, and cell culture supernatant samples. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.
The analysis should be performed by trained laboratory professionals.

## BACKGROUND

Fatty Acid Binding Proteins (FABPs), are a family of proteins with an affinity for long-chain fatty acids (LCFAs) and other fat soluble molecules. These small intracellular proteins are abundantly expressed in a highly tissue-specific manner. FABPs are known to be critical for fatty acid uptake and intracellular transport, but they are also important for regulation of lipid metabolism and cellular signaling. These functions point to the role that FABPs play as cytoprotectants to fatty acid oxidative stressors.

Specifically, Liver Fatty Acid Binding Protein (L-FABP), also called FABP1 (or FABP-1), is a 14 kDa protein that is predominantly expressed in the liver. It comprises 2-11% of the cytosolic protein of normal hepatocytes. It is also expressed in tubular kidney cells, the alveolar epithelium of the lung and in enterocytes of the small intestine.

## SUMMARY AND EXPLANTION OF TEST

The Diapharma Human L-FABP ELISA is a solid-phase sandwich enzyme-linked immunoassay with an assay time of 3.5 hours. The kit offers flexibility in sample or batch size with 12 disposable 8-well strips. Standards and samples are pipetted into the wells which are pre-coated with a monoclonal antibody directed to human L-FABP. In a second step, a biotinylated detection antibody binds to the captured human L-FABP. A streptavidin-peroxidase conjugate is added which binds to the biotinylated detection antibody. TMB Substrate is added and reacts with the streptavidin-peroxidase conjugate. The enzyme reaction is stopped by the addition of oxalic acid. A spectrophotometer is used to read absorbance at 450 nm. The measured absorbance is directly proportional to the concentration of the human L-FABP antigen.

## MATERIALS FOR 96 DETERMINATIONS

Each Kit includes:

| | |
|---|---|
| Wash buffer 40x | 1 vial (30 ml) |
| Dilution buffer 10x | 1 vial (15 ml) |
| Standard | 2 vials, lyophilized |
| Detection Ab, biotinylated | 1 vial, 1 ml lyophilized |
| Streptavidin-peroxidase 100x | 1 tube, 0.25 ml in solution |
| TMB substrate | 1 vial (11 ml) |
| Stop solution | 1 vial (22 ml) |
| 12 Microtiter strips, pre-coated | 1 plate |
| Certificate of Analysis | 1 |

## STORAGE AND SHELF LIFE AFTER FIRST OPENING

- Upon receipt, store individual components at 2 - 8°C. Do not freeze.
- Do not use components beyond the expiration date printed on the kit label.
- The standard and detection antibody are provided in lyophilized form and is stable until the expiration date indicated on the label, if stored at 2-8°C
- The streptavidin-peroxidase is provided in concentrated solution and is stable until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact amount of the standard is indicated on the label of the vial and the Certificate of Analysis.
- The standard is single use. The standard cannot be stored for repeated use.
- Once reconstituted the detection antibody is stable for 1 month if stored at 2 - 8°C.
- The streptavidin-peroxidase can only be stored in concentrated solution and is not stable when stored diluted.
- Upon receipt, foil pouch around the plate should be vacuum-sealed and unpunctured. Any irregularities to aforementioned conditions may influence plate performance in the assay.
- Return unused strips immediately to the foil pouch containing the desiccant pack and reseal along the entire edge of the zip-seal. Quality guaranteed for 1 month if stored at 2 - 8°C.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Calibrated micropipettes and disposable tips.
- Distilled or de-ionized water.
- Plate washer: automatic or manual.
- Polypropylene tubes.
- Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.
- Adhesive covers can be ordered separately. Please contact your local distributor.
- Centrifuge for 1 ml tubes.

## ASSAY PROTOCOL

## WARNINGS AND PRECAUTIONS

- For research use only, not for diagnostic or therapeutic use.
- This kit should only be used by qualified laboratory staff.
- Do not use kit components beyond the expiration date.
- Do not ingest any of the kit components.
- Do not mouth pipet any of the kit components.
- Kit reagents contain 2-chloroacetamide as a preservative. 2-Chloroacetamide is harmful in contact with skin and toxic if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
- The TMB substrate is light sensitive, keep away from bright light. The solution should be colorless until use.
- The stop solution contains 2% oxalic acid and can cause irritation or burns to respiratory system, skin and eyes. Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advice.
- Handle all biological samples as potentially hazardous and capable of transmitting diseases.
- Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.

- The standard is of human origin. It was tested for various viruses and found negative. Since no test method can offer complete assurance that infectious agents are absent, this reagent should be handled as any potentially infectious human serum or blood specimen. Handle all materials in contact with this reagent according to guide-lines for prevention of transmission of blood-borne infections.

## SAMPLE PREPARATION

### Serum or plasma

Collect blood using normal aseptic techniques. Blood samples should be kept on ice. If serum is used, separate serum from blood after clotting at room temperature within 1 hour by centrifugation (1500xg at 4°C for 15 min). Transfer the serum to a fresh polypropylene tube.
If plasma is used, separate plasma from blood within 20 minutes after blood sampling by centrifugation (1500xg at 4°C for 15 min). Transfer the plasma to a fresh polypropylene tube.
Most reliable results are obtained if EDTA plasma is used.

### Urine

Collect urine using normal aseptic techniques. Centrifuge the urine to remove debris (1500xg at 4 °C for 15 min). Transfer urine to a fresh polypropylene tube.

### Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of human L-FABP. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of human L-FABP activity and give erroneous results.
Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples.
Before performing the assay, samples should be brought to room temperature (18 – 25°C) and mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

## DILUTION PROCEDURES

### Serum or plasma samples

Human L-FABP can be measured accurately if serum or plasma samples are diluted at least 20x before use with supplied dilution buffer in polypropylene tubes. Note that most reliable results are obtained with EDTA plasma.

### Urine samples

Human L-FABP can be measured accurately if urine samples are diluted at least 20x with supplied dilution buffer in polypropylene tubes.

### Sample dilution

The mentioned dilution for samples is a minimum dilution and should be used as a guideline. The recovery of human L-FABP from an undiluted sample is not 100% and may vary from sample to sample. When testing less diluted samples it is advisable to run recovery experiments to determine the influence of the matrix on the detection of human L-FABP.
Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.
Sample volumes should total at least 230 µl of diluted sample, which is sufficient for one sample in duplicate in the ELISA. For dilution of samples we recommend to use at least 10 µl of sample.

## COMPONENT PREPARATION

- Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.
- Open vials carefully: vials are under vacuum.
- Do not mix reagents from different kits and lots. The reagents have been standardized as a unit for a given lot. Use only the reagents supplied by the manufacturer.
- Do not under any circumstances add sodium azide as preservative to any of the components.
- Do not reuse microwells or pour reagents back into their bottles once dispensed.

### Wash buffer

Prepare wash buffer by mixing 30 ml of 40x wash buffer with 1170 ml of distilled or de-ionized water.

### Dilution buffer

Prepare dilution buffer by mixing 15 ml of the 10x dilution buffer with 135 ml of distilled or de-ionized water. Concentrated dilution buffer may contain crystals. In case the crystals do not disappear at room temperature within 1 hour, concentrated dilution buffer can be warmed up to 37°C. Do not shake the solution.

### Standard solution

The standard is reconstituted by pipetting the amount of dilution buffer mentioned on the CoA in the standard vial. Prepare each L-FABP standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in the below schematic. ***After reconstitution the standard cannot be stored for repeated use.*** The assay has been optimized for the indicated standard range. Do not change the standard range. Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.

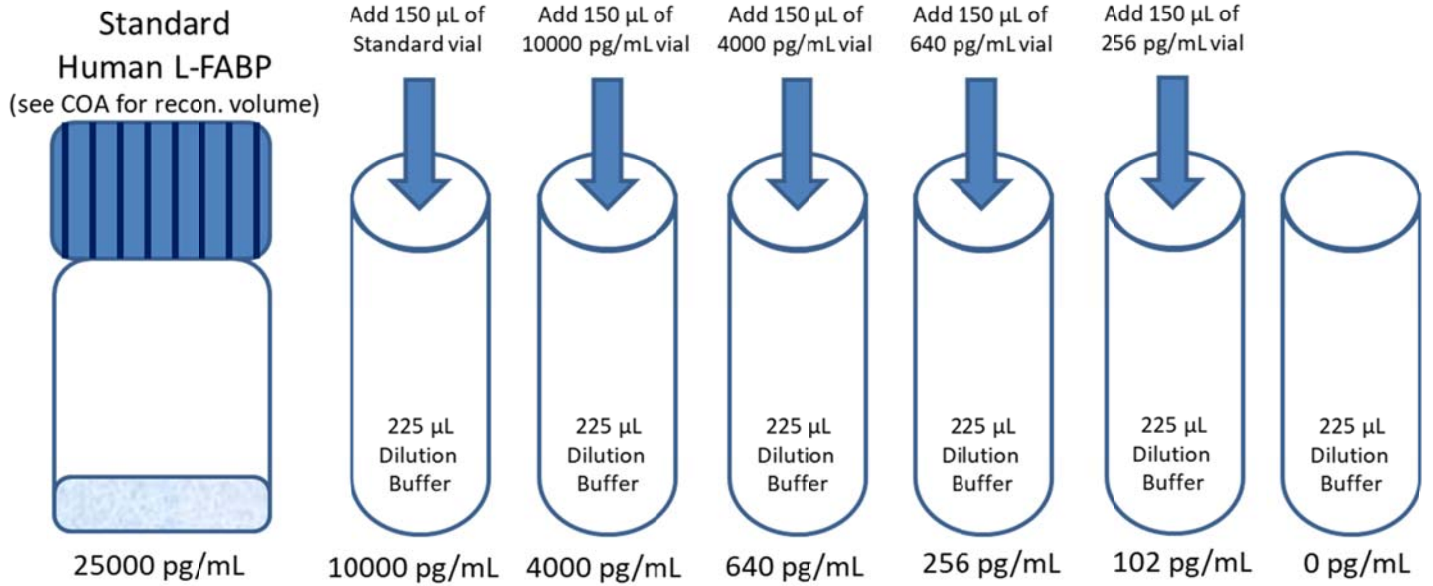
### Detection antibody solution

The detection antibody is reconstituted by pipetting 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml detection antibody with 11 ml dilution buffer.

### Streptavidin-peroxidase solution

Spin down streptavidin-peroxidase tubes before use. Prepare the streptavidin-peroxidase solution by mixing 0.25 ml of the 100x streptavidin-peroxidase solution with 24.75 ml dilution buffer.

# Standard Reconstitution and Dilution Schematic



## ASSAY PROCEDURE

Bring all reagents to room temperature (20 - 25°C) before use.

1. Determine the number of test wells required, put the necessary microwell strips into the supplied frame. Return the unused strips to the storage bag with desiccant, seal and store at 2 - 8°C.

2. Transfer 100 µl in duplicate of standard, samples, or controls into appropriate wells. Do not touch the side or bottom of the wells.

3. Cover the tray. Tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.

4. Incubate the strips or plate for 1 hour at room temperature.

5. Wash the plates 4 times with wash solution as follows:

    a. Carefully remove the cover, avoid splashing.

    b. Empty the plate by inverting plate and shaking contents out over the sink, keep inverted and tap dry on a thick layer of tissues.

    c. Add 200 µl of wash buffer to each well, wait 20 seconds, empty the plate as described in 5b.

    d. Repeat the washing procedure 5b/5c three times.

    e. Empty the plate and gently tap on thick layer of tissues.

6. Add 100 µl of diluted detection antibody to each well using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.

7. Cover the tray and incubate the tray for 1 hour at room temperature.

8. Repeat the wash procedure described in step 5.

9. Add 100 µl of diluted streptavidin-peroxidase to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.

10. Cover the tray and incubate the tray for 1 hour at room temperature.

11. Repeat the wash procedure described in step 5.

12. Add 100 µl of TMB substrate to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.

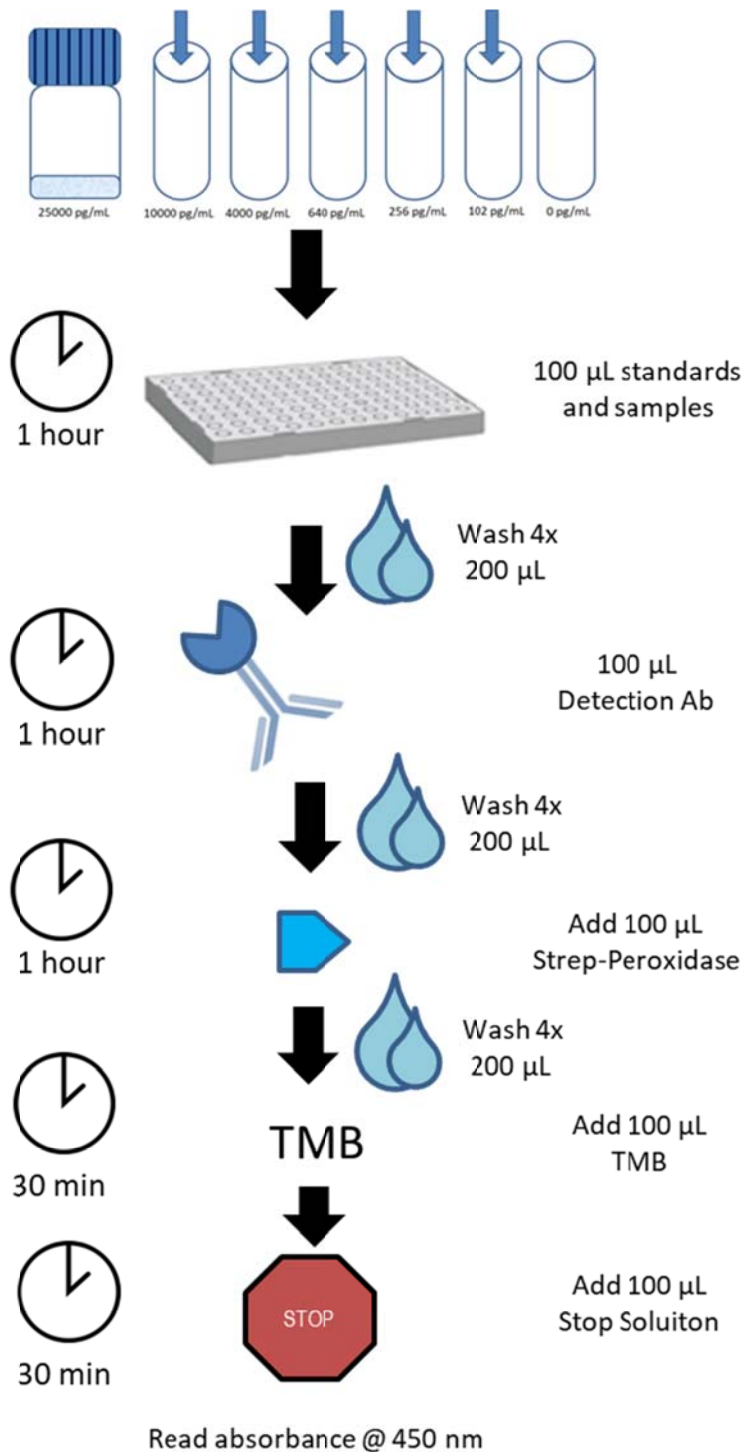
13. Cover the tray and incubate the tray for 30 minutes at room temperature. It is advised to control the reaction on the plate regularly. In case of strong development the TMB reaction can be stopped sooner. Avoid exposing the microwell strips to direct sunlight. Covering the plate with aluminum foil is recommended.

14. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 12. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.

15. Read the plate within 30 minutes after addition of stop solution at 450 nm using a plate reader, following the instructions provided by the instrument’s manufacturer.

*Note that incubation times and pipetting volumes other than those specified may give erroneous results.

*Note that automated plate washer protocols should be validated against manual protocols.




## CALCULATION OF ANALYTICAL RESULTS

- Calculate the mean absorbance for each set of duplicate standards, control and samples.
- If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be retested.
- The mean absorbance of the zero standard should be less than 0.3.

- Create a standard curve using computer software capable of generating a good curve fit. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis (logarithmic scale).
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Samples that give a mean absorbance above the absorbance for the highest standard concentration are out of range of the assay. These samples should be retested at a higher dilution.

**PERFORMANCE**

Measuring range: The minimum concentration which can be measured is 102 pg/ml. The measuring range is 102 – 25,000 pg/ml.

Spiking Recovery: Normal human blood samples (plasma), containing baseline levels of human L-FABP, were spiked with human L-FABP in concentrations of 0.2 and 5 ng/mL. Samples plus and minus L-FABP were incubated for 1 hour at room temperature. Samples were measured using the ELISA. Values for L-FABP ranged between 86% and 102% (mean 94%).

Reference range: Serum/plasma and urine of healthy individuals contains approximately 12 ng/ml and 16 ng/ml, respectively.

Cross Reactivity:

| **Cross reactant** | **Reactivity** |
|---|---|
| Rhesus Monkey L-FABP | Positive |
| Swine L-FABP | Positive* |
| Human H-FABP | Negative |
| Human I-FABP | Negative |
| Mouse/Rat L-FABP | Negative |

* In order to determine the results for swine, calculated concentrations should be multiplied with 1.8.

## TECHNICAL HINTS

- In case plate washer is used, please note: use of a plate washer can result in higher background and decrease in sensitivity. We advise validation of the plate washer with the manual procedure.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every run. Standards should be referred to the standard curve prepared on the same plate.
- Do not mix reagents from different batches, or other reagents and strips. Remainders should not be mixed with contents of freshly opened vials.

- Each time the kit is used, fresh dilutions of standard, sample, detection antibody, streptavidin-peroxidase and buffers should be made.
- If user is not familiar with the ELISA technique it is recommended that the user perform a pilot assay prior to evaluation of your samples. Perform the assay with a standard curve only following the instructions.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing wash buffer, fill with wash buffer as indicated for each cycle and do not allow wells to sit uncovered or dry for extended periods.
- To avoid cross-contaminations, change pipette tips between reagent additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Waste disposal should be performed according to your laboratory regulations.

Do not hesitate to contact our technical support team at techsupport@diapharma.com.