EAGLE BIOSCIENCES

Mouse H-FABP ELISA Kit

Catalog Number:
MFP11-K01 (1 x 96 wells)
MFP11-K02 (2 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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INTENDED USE

The Eagle Biosciences Mouse H-FABP ELISA Kit is to be used for the quantitative determination of mouse H-FABP in serum or plasma samples. The Eagle Biosciences Mouse H-FABP ELISA Kit is for research use only and not for diagnostic or therapeutic procedures.

INTRODUCTION

Fatty acid-binding proteins (FABPs) are a class of cytoplasmic proteins that bind long chain fatty acids. FABPs are small intracellular proteins (~13-14 kDa) with a high degree of tissue specificity. They are abundantly present in various cell types and play an important role in the intracellular utilization of fatty acids, transport and metabolism. There are at least nine distinct types of FABP, each showing a specific pattern of tissue expression. Due to its small size, FABP leaks rapidly out of ischemically damaged necrotic cells leading to a rise in serum levels. Ischemically damaged tissues are characterized histologically by absence (or low presence) of FABP facilitating recognition of such areas.

The H-FABP protein is derived from the FABP3 gene. The FABP content of mouse heart muscle (H-FABP) is markedly high, 10-20 mol% of cytoplasmic proteins. Following acute myocardial infarction (AMI) the small protein H-FABP is rapidly released into the circulation. Significantly elevated serum/plasma concentrations are found within 3 hours after AMI which generally return to normal values within 12 to 24 hours. These features make H-FABP a useful research tool for the early assessment or exclusion of AMI, and for the monitoring of a recurrent infarction. Constitutive H-FABP released from the heart after AMI is quantitatively recovered in serum/plasma. Thus assessment of H-FABP is also a very effective tool for the estimation of the infarct size. The mouse H-FABP kit can also be used for measurement of brain-type FABP, a marker for brain injury detection.

In serum/plasma of healthy human individuals approximately 1.6 ng/ml of H-FABP is present.

PRINCIPLE OF THE ASSAY

The mouse H-FABP ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3½ hours for Mouse H-FABP. The efficient format of 2 plates with twelve disposable 8-well strips allows free choice of batch size for the assay. Samples and standards are captured by a solid bound specific antibody. Biotinylated tracer antibody will bind to captured mouse H-FABP. Streptavidin-peroxidase conjugate will bind to the biotinylated tracer antibody. Streptavidin-peroxidase conjugate will react with the substrate, tetramethylbenzidine (TMB). The enzyme reaction is stopped by the addition of citric acid. The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the mouse/rat H-FABP standards (log). The H-FABP concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

KIT FEATURES

- Working time of 3 ½ hours.
- Minimum concentration which can be measured is 391 pg/ml.
- Measurable concentration range of 391 to 25,000 pg/ml.
- Working volume of 100 µl/well.

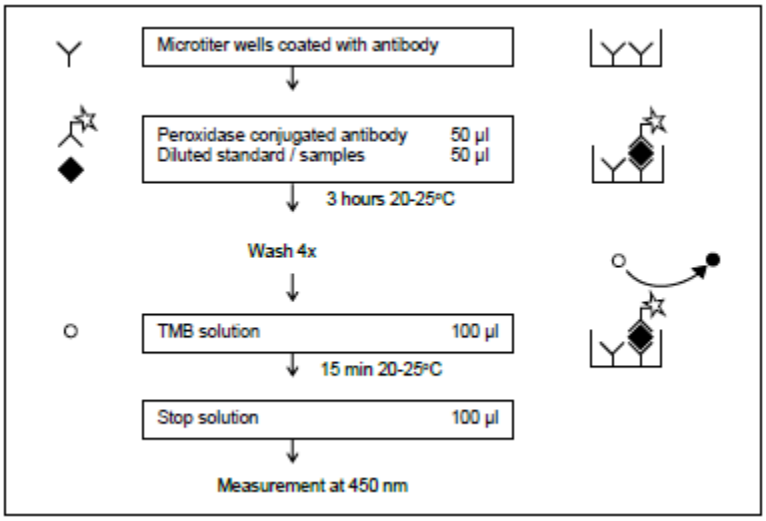
CROSS REACTIVITY

Potential cross-reacting proteins detected in the mouse H-FABP ELISA:

Cross reactant	Reactivity
Swine H-FABP	Strong
Human H-FABP	Strong
Rat/Mouse I-FABP	Negative
Rat/Mouse L-FABP	Negative
Rat H-FABP	Strong

Cross-reactivity for other species or proteins/peptides has not been tested.

PROTOCOL OVERVIEW



- The mouse H-FABP ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3¼ hours.
- The efficient format of a plate with twelve disposable 8-well strips allows free choice of batch size for the assay.
- Samples and standards are incubated together with peroxidase-conjugated second antibody in microtiter wells coated with antibodies recognizing mouse H-FABP.
- During incubation mouse H-FABP is captured by the solid bound antibody. The secondary antibodies will bind to the captured mouse H-FABP.
- The peroxidase conjugated antibody will react with the substrate, tetramethylbenzidine (TMB).
- The enzyme reaction is stopped by the addition of oxalic acid.
- The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the mouse H-FABP standards (log).
- The mouse H-FABP concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

KIT COMPONENTS AND STORAGE INSTRUCTIONS

Kit Component	Quantity MFP11-K01	Quantity MFP11-K02	Color Code
Wash buffer 20x	1 vial (60 ml)	1 vial (60 ml)	Colorless
Dilution Buffer 10x	1 vial (15 ml)	1 vial (15 ml)	Green
Standard	2 vials, lyophilized	4 vials, lyophilized	White
Conjugate, peroxidase-labeled	1 vial, 1 ml lyophilized	2 vials, 1 ml lyophilized	Blue
TMB substrate	1 vial (11 ml)	1 vial (22 ml)	Brown
Stop solution	1 vial (22 ml)	1 vial (22 ml)	Red
12 Microtiter strips, pre-coated	1 plate	2 plates	
Certificate of Analysis	1	1	
Manual	1	1	
Data collection sheet	2	2	

- 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 conjugate are stable in lyophilized form until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The standard is single use. After reconstitution the standard cannot be stored.
- The exact amount of the standard is indicated on the label of the vial and the Certificate of Analysis.
- Once reconstituted the conjugate is stable for 1 month if stored at 2 - 8°C.
- 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.

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 under any circumstances add sodium azide as preservative to any of the components.
- Do not use kit components beyond the expiration date.
- 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 manufacturer.
- The assay has been optimized for the indicated standard range. Do not change the standard range.
- Open vials carefully: vials are under vacuum.
- It is advised to spin down streptavidin-peroxidase tubes before use.

- Do not ingest 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.
- Incubation times, incubation temperature and pipetting volumes other than those specified may give erroneous results.
- Do not reuse micro wells or pour reagents back into their bottles once dispensed.
- Handle all biological samples as potentially hazardous and capable of transmitting diseases.
- Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.
- Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.

SAMPLE PREPARATION

Collection and handling

Serum or plasma
Collect blood using normal aseptic techniques. 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.

Storage
Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of mouse H-FABP. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of mouse H-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
Mouse H-FABP can be measured accurately if serum or plasma samples are diluted at least 2x with supplied Dilution buffer in polypropylene tubes.
Most reliable results are obtained if EDTA plasma is used.

Comment regarding recommended sample dilution
The mentioned dilution for samples is a minimum dilution and should be used as a guideline. The recovery of mouse H-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 mouse H-FABP.

Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

Guideline for dilution of samples

Please see table 2 for recommended for sample dilutions. Volumes are based on a total volume of 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.

Dilution	Pre-Dilution	Amount of sample or pre-dilution required	Amount of dilution buffer required
10x	Not necessary	25 µl (sample)	225 µl
20x	Not necessary	15 µl (sample)	285 µl
50x	Not necessary	10 µl (sample)	490 µl
100x	Not necessary	10 µl (sample)	990 µl
500x	Recommended: 10x (see nr. 1)	10 µl (pre-dilution)	490 µl
1000x	Recommended: 10x (see nr. 1)	10 µl (pre-dilution)	990 µl
2000x	Recommended: 20x (see nr. 2)	10 µl (pre-dilution)	990 µl
5000x	Recommended: 50x (see nr.3)	10 µl (pre-dilution)	990 µl

REAGENT PREPARATION

Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.

Wash buffer

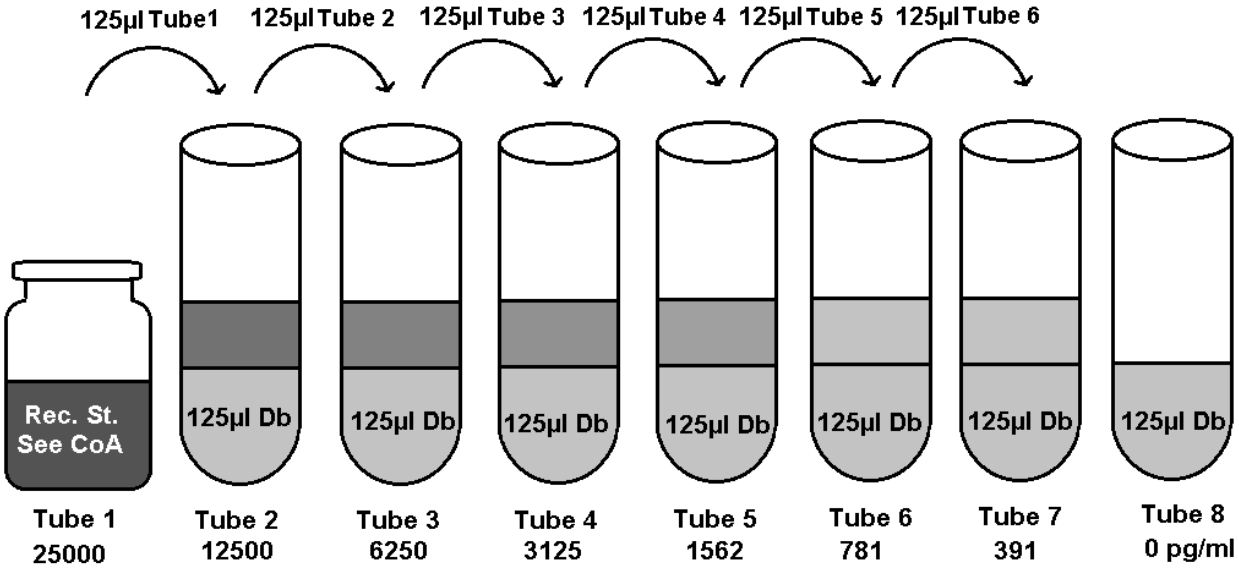
Prepare wash buffer by mixing 60 ml of 20x wash buffer with 1140 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 20x wash buffer with 19 parts of distilled or de-ionized water.

Dilution buffer

Prepare dilution buffer by mixing 15 ml of the 10x dilution buffer with 135 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of dilution buffer by diluting 1 part of the 10x dilution buffer with 9 parts 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. Use the standard vial as Tube 1 in Figure 1. Prepare each mouse H-FABP standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in Figure 1*. After reconstitution the standard must be used within 1 hour and cannot be stored for repeated use.



Conjugate solution
The conjugate is reconstituted by pipetting 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml conjugate with 5 ml dilution buffer, which is sufficient for 1 x 96 tests. Where less volume is required, prepare the desired volume of conjugate by diluting 1 part of the reconstituted tracer with 5 parts of dilution buffer.

ELISA PROTOCOL
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, and fill out the data collection sheet. Return the unused strips to the storage bag with desiccant, seal and store at 2 - 8°C.
2. Add 50 µl of diluted conjugate to each well. Do not touch the side or bottom of the wells.
3. Transfer 50 µl in duplicate of standard, samples, or controls into appropriate wells. Do not touch the side or bottom of the wells.
4. Cover the tray and tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
5. Incubate the strips or plate for 3 hours at room temperature for mouse samples.
6. Wash the plates 4 times with wash buffer using a plate washer or 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 6b.
    d. Repeat the washing procedure 6b/6c three times.
    e. Empty the plate and gently tap on thick layer of tissues.
7. Add 100 µl of TMB substrate to each well. Do not touch the side or bottom of the wells.
8. Cover the tray and incubate the tray for 15 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 aluminium foil is recommended.

9. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 7. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
10. 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.

*) 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.
Make sure the plate washer is used as specified for the manual method.

INTERPRETATION OF 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.

TECHNICAL HINTS

- User should be trained and familiar with ELISA assays and test procedure.
- If you are not familiar with the ELISA technique it is recommended to 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.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every run. Samples 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, tracer, streptavidin-peroxidase and buffers should be made.
- Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.
- 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.
- The waste disposal should be performed according to your laboratory regulations.

REFERENCES

1. Aartsen, W et al; Heart fatty acid binding protein and cardiac troponin T plasma concentrations as markers for myocardial infarction after coronary artery ligation in mice. Eur J Physiol 2000, 439: 416
2. Van der Lee, K et al; Fasting-induced changes in the expression of genes controlling substrate metabolism in the rat heart. J Lipid Res 2001, 42: 1752
3. Verges, L et al; Heart-type Fatty Acid-binding Protein Is Essential for Efficient Brown Adipose Tissue Fatty Acid Oxidation and Cold Tolerance. J of Biol Chem 2011, 286:380

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