



HK401

HUMAN H-FABP

ELISA KIT

PRODUCT INFORMATION & MANUAL

Read carefully prior to starting procedures!

ATTENTION

For use in laboratory research only
Not for clinical or diagnostic use

Note that this user protocol is not lot-specific and is representative for the current specifications of this product. Please consult the vial label and the certificate of quality control for information on specific lots. Also note that shipping conditions may differ from storage conditions.

For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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1. INTENDED USE

The human H-FABP ELISA kit is to be used for the *in vitro* quantitative determination of human H-FABP in serum and plasma samples. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

The kit is presented in a two assay format. The normal format, for more than 40 determinations, takes about 1½ hours. The rapid format takes about 50 minutes.

The analysis should be performed by trained laboratory professionals.

2. 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.

Following acute myocardial infarction (AMI) the small protein H-FABP is rapidly released into the circulation. H-FABP is derived from the human *FABP3* gene. Significantly elevated serum/plasma concentrations are found within 3 h after AMI which generally return to normal values within 12 to 24 h. 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 human H-FABP kit can also be used for measurement of brain-type FABP, a marker for brain injury detection and for measurement of muscle-type cytosolic fatty acid binding protein (FABPc) in skeletal muscle.

In serum/plasma of healthy individuals approximately 1.6 ng/ml of H-FABP is present. H-FABP shows a slight increase with age.

3. KIT FEATURES

- Working time of 1½ hours (normal) or ¾ (rapid) hour.
- Minimum concentration which can be measured is 250 pg/ml.
- Measurable concentration range of 100 to 25,000 pg/ml.
- Working volume of 100 µl/well.

Cross-reactivity

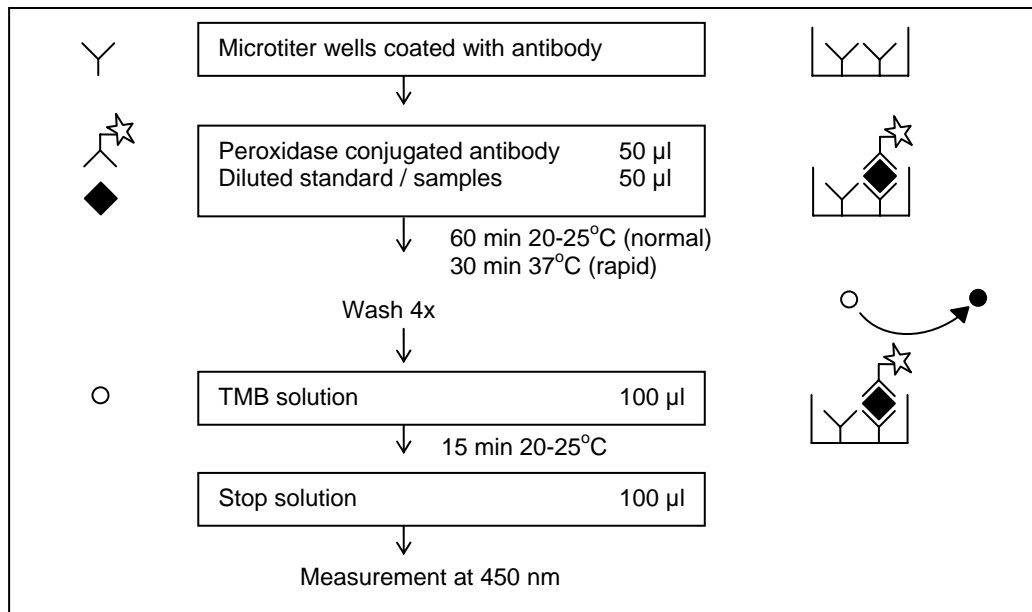
Potential cross-reacting proteins detected in the human H-FABP ELISA:

Cross reactant	Reactivity
Human I-FABP	Negative
Human L-FABP	Negative
Swine H-FABP	Average
Horse H-FABP	Average
Salmon H-FABP	Average

Table 1

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

4. PROTOCOL OVERVIEW



- The human H-FABP ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 1½ (normal) or ¾ (rapid) hours.
- The efficient format of 1 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 human H-FABP.
- During incubation human H-FABP is captured by the solid bound antibody. The secondary antibodies will bind to the captured human 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 human H-FABP standards (log).
- The human H-FABP concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

5. KIT COMPONENTS AND STORAGE INSTRUCTIONS

Item no.	Kit component	Quantity	Color code
Vial 1	Wash buffer 20x	1 vial (20 ml)	Grey
Vial 2	Dilution buffer 10x	1 vial (10 ml)	Gold
Vial 3	Standard	1 vial, 1 ml lyophilized	Yellow
Vial 4	Conjugate, peroxidase-labeled	1 vial, 1 ml lyophilized	Blue
Vial 5	TMB substrate	1 vial (20 ml)	Purple
Vial 6	Stop solution	1 vial (20 ml)	Red
Item 7	12 Microtiter strips, pre-coated	1 plate	
Item 8	Frame	1	
Item 9	Adhesive covers	4	
Item 10	Certificate of quality control	1	
Item 11	Manual	1	
Item 12	Data collection sheet	1	

Table 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 exact concentration of the standard is indicated on the label of the vial and the certificate of quality control.
- Once reconstituted, standard and conjugate are 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 until expiration date 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.
In case a plate washer is used the supplied wash buffer is not sufficient. Additional wash buffer can be ordered separately. Please contact your local distributor.
- Polypropylene tubes.
- Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.

6. 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.
- Standard and conjugate vials should be opened after reconstitution. Open vials carefully: vials are under vacuum.
- 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 microwells 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.

7. 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 heparin or 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 species protein. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of species protein 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 H-FABP can be measured accurately if serum or plasma samples are diluted at least 5x with supplied dilution buffer. in polypropylene tubes

Most reliable results are obtained if heparin or EDTA plasma is used.

Remark regarding recommended sample dilution

The recommend dilution for samples should be used as a guideline. The recovery of species protein 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 species protein.

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

8. 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 20 ml of 20x wash buffer with 380 ml of distilled or de-ionized water, which is enough for 1 x 96 tests. Where 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 10 ml of the 10x dilution buffer with 90 ml of distilled or de-ionized water, which is sufficient for 1 x 96 tests. Where 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 injection of 1 ml of distilled or de-ionized water. Prepare each human H-FABP standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in Table 3.

Tube	Volume dilution buffer	Volume standard	Concentration (pg/ml)
1	See certificate of quality control	100 µl vial 3	25000
2	150 µl	100 µl tube 1	10000
3	150 µl	100 µl tube 2	4000
4	150 µl	100 µl tube 3	1600
5	150 µl	100 µl tube 4	640
6	150 µl	100 µl tube 5	256
7	150 µl	100 µl tube 6	102
8	150 µl	-	0

Table 3

Conjugate solution

The conjugate is reconstituted by injection of 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 conjugate with 5 parts of dilution buffer.

9. 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.
3. Transfer 50 µl in duplicate of standard, samples, or controls into appropriate wells.
4. Apply an adhesive cover to the tray. Tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
5. Incubate the strips or plate for 60 minutes at room temperature for normal or 30 minutes at 37°C for rapid format.
6. Wash the plates 4 times with wash buffer using a plate washer or as follows:
 - a. Carefully remove the plate sealer, 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 with a new adhesive cover, incubate the tray for 15 minutes at room temperature. 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.

10. 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). For an example of the standard curve see certificate of quality control included with the kit. If the standard is out of range, the results of the test samples are not reliable. The test should be repeated.
- 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.

11. 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. If the standard is out of range, the results of the test samples are not reliable. The test should be repeated.
- 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, conjugate 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.
- To ensure accurate results, proper adhesion of supplied covers during incubation steps is necessary.
- The waste disposal should be performed according to your laboratory regulations.

Technical support

Do not hesitate to contact our technical support team at support@hycultbiotech.com for inquiries and technical support regarding the human H-FABP ELISA.

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12. QUALITY CONTROL

The certificate of quality control included in this kit is lot specific and is to be used to verify results obtained by your laboratory. The absorption values provided on the certificate of quality control are to be used as a guideline only. The results obtained by your laboratory may differ.

This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Hycult Biotech immunoassay, the possibility of interference cannot be excluded.

For optimal performance of this kit, it is advised to work according to good laboratory practice.

13. TROUBLESHOOTING

Warranty claims and complaints in respect of deficiencies must be logged before expiry date of the product. A written complaint containing lot number of the product and experimental data should be sent to support@hycultbiotech.com.

Suggestions summarized below in Table 4 can be used as a guideline in the case of unexpected assay results.

Low absorbance	High absorbance	Poor duplicates	All wells positive	All wells negative	Possible cause
•	•		•	•	Kit materials or reagents are contaminated or expired
•					Incorrect reagents used
•		•	•		Lyophilized reagents are not properly reconstituted
•	•	•	•	•	Incorrect dilutions or pipetting errors
•		•			Improper plastics used for preparation of standard and/or samples
•	•				Improper incubation times or temperature
		•			Especially in case of 37°C incubation: plates are not incubated uniformly
•					Assay performed before reagents were brought to room temperature
•	•	•	•	•	Procedure not followed correctly
				•	Omission of a reagent or a step
		•			Poor mixing of samples
	•		•		Low purity of water
	•	•			Strips were kept dry for too long during/after washing
	•	•	•		Inefficient washing
	•	•			Cross-contamination from other samples or positive control
		•	•		TMB solution is not clear or colorless
•	•				Wrong filter in the microtiter reader
	•	•			Airbubbles
		•			Imprecise sealing of the plate after use
•					Wrong storage conditions

Table 4

14. REFERENCES

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5. Morariu, A et al; Dexamethasone: benefit and prejudice for patients undergoing on-pump coronary artery bypass grafting: a study on myocardial, pulmonary, renal, intestinal, and hepatic injury. *Chest* 2005, *128*: 2677