

Reagent for quantification of human heart-type fatty acid-binding protein (H-FABP) in serum or plasma

MARKIT-M H-FABP

■ Introduction

Heart-type fatty acid-binding protein (H-FABP) is a soluble cytoplasmic protein with a low molecular weight of 14.9 kDa which is considered to be the key fatty acid carrier protein. It is especially abundant in the myocardium. H-FABP is immunologically distinguishable from other types of FABP, such as intestinal-type FABP (I-FABP) and liver-type FABP (L-FABP). Because H-FABP is readily released into the circulation in the early phase of myocardial injury, it was proposed that H-FABP may be an early marker for detection of acute myocardial infarction (AMI). Dr. Tanaka's group (Osaka Medical College) reported that H-FABP showed excellent diagnostic sensitivity even in the superacute phase within 3 hrs after the onset of symptoms, and for clinical assessment 6.2 ng of H-FABP per mL of serum showed the highest diagnostic efficacy. In cooperation with Dr. Tanaka, we have succeeded in developing a sandwich-enzyme-linked immunosorbent assay (ELISA), MARKIT-M H-FABP, using two distinct mouse anti-human H-FABP monoclonal antibodies. This ELISA can measure the concentration of H-FABP in serum and/or plasma within 75 min and can be used for the diagnosis of AMI.

■ Contents of MARKIT-M H-FABP

Each kit (96 tests) contains the following reagents.

Standard 0 (lyophilized): 1 vial (for 2.0 mL).

Standard 5 (lyophilized): 1 vial (for 0.5 mL) contains 2.5 ng of H-FABP*.

Standard 10 (lyophilized): 1 vial (for 0.5 mL) contains 5 ng of H-FABP*.

Standard 25 (lyophilized): 1 vial (for 0.5 mL) contains 12.5 ng of H-FABP*.

Standard 50 (lyophilized): 1 vial (for 0.5 mL) contains 25 ng of H-FABP*.

Standard 100 (lyophilized): 1 vial (for 0.5 mL) contains 50 ng of H-FABP*.

Standard 250 (lyophilized): 1 vial (for 0.5 mL) contains 125 ng of H-FABP*.

H-FABP antibody-coated wells: 1 plate (96 wells). Each well contains: anti-human H-FABP monoclonal antibody (mouse).

Buffer solution (bottle No. 1): 1 bottle (10 mL).

For research use only

Wash buffer concentrate (bottle No. 2): 3 bottles (30 mL each).

H-FABP antibody-enzyme conjugate (bottle No. 3): 1 bottle (15 mL), containing HRP-labeled anti-human H-FABP monoclonal antibody (mouse).

Substrate tablets: 3. Each tablet contains o-phenylenediamine dihydrochloride (OPD) (13 mg).

Substrate diluent buffer (bottle No. 4): 3 bottles (15 mL each). Each bottle contains hydrogen peroxide (15 μ L).

Stop solution (bottle No. 5): 1 bottle (15 mL).

Microplate for dilution: 1 plate (96 wells).

Graph paper: 3 sheets.

*H-FABP: Human H-FABP is quantitatively determined by an immunological method using purified human H-FABP as a standard material.

■ Application

Quantification of H-FABP in human serum or plasma.

■ Characteristics

- (1) MARKIT-M H-FABP is for the quantification of H-FABP in human serum or plasma based on a direct sandwich-ELISA.
- (2) This assay is highly specific for human H-FABP because two distinct mouse anti-human H-FABP monoclonal antibodies are used.
- (3) Co-existing components and related substances in blood do not influence the quantification of H-FABP with this kit.
- (4) MARKIT-M H-FABP is a rapid and convenient assay kit: many samples can be assayed at the same time.

■ Principle

Two-step direct sandwich ELISA using two distinct mouse anti-human H-FABP monoclonal antibodies.

■ Assay method

1. Instruments and materials required

Pipettes (with disposable tips) of 70, 500 and 2000 μ L, multichannel pipettes of 100 and 300 μ L, volumetric cylinder of 500 mL, microplate mixer, ELISA washer, microplate reader equipped with 492 nm (as the main wave length) and 620 nm (as

the reference wave length) detectors, stopwatch, paper towels, aluminum foil, etc.

2. Preparation of sample

- (1) Use serum or plasma as the sample for assay.
- (2) In the case of plasma samples, use blood collection tubes containing heparin or EDTA.
- (3) In the case of storage of serum or plasma samples, keep them frozen at -20°C or lower.
- (4) If the sample contains a high concentration of H-FABP (more than 250 ng/mL), the sample should be diluted with standard solution 0 for the assay.

3. Preparation of reagents

(1) Standard solutions

Accurately add 2.0 mL and 0.5 mL of purified water to the standard 0 vial and other standard reagent vials, respectively. Stand for 15 min and then shake the vials gently to thoroughly dissolve the contents. (Standard solutions are stable for at least 1 week at $2-10^{\circ}\text{C}$ or at least 1 month at -20°C . No influence on the stability was observed after 5 cycles of freezing-thawing.)

(2) H-FABP antibody-coated wells

Cut and remove the seal adhering to the number of wells you need. Then discard the preservation liquid in each well. Turn the wells upside down and tap on a paper towel to remove the preservation liquid*. The rest of the strip wells should be preserved at $2-10^{\circ}\text{C}$ until use for another assay.

*Note: Never dry the wells completely.

(3) Wash buffer

Put the whole volume (30 mL) of the wash buffer concentrate (bottle No. 2) into a 500-mL volumetric cylinder and dilute with purified water to 300 mL. Use this as a wash buffer. (This wash buffer is stable for at least 1 week at $15-25^{\circ}\text{C}$.)

(4) Substrate solution

Place one substrate tablet (OPD) in one bottle of substrate diluent buffer (bottle No. 4), mix gently and use it as the substrate solution. Prepare the substrate solution just 30 min before use, and keep it shielded from light.

4. Procedure

See the following methods and figure.

It is preferable to determine standard solutions in duplicate.

Before starting the assay, adjust samples and reagents to $15-25^{\circ}\text{C}$.

Keep the H-FABP antibody-coated wells horizontal during the assay.

- (1) Place 70 μL of buffer solution in each well of the microplate for dilution.

Then add 70 μ L of standard solution or sample to the wells. Agitate the microplate gently on a microplate mixer.

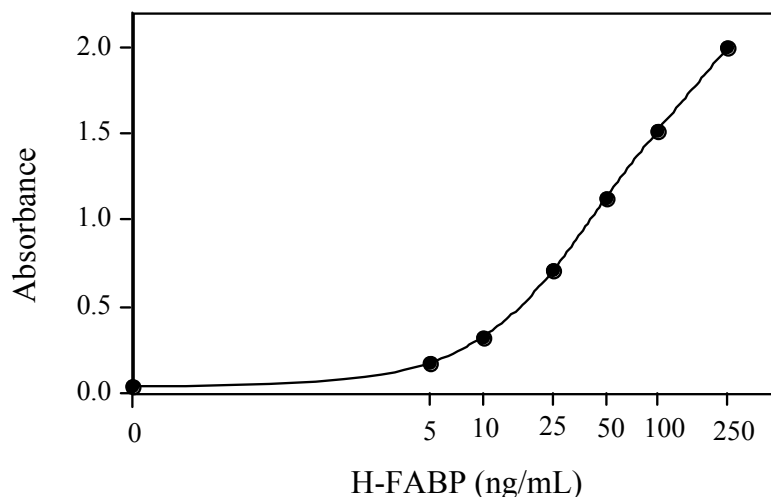
- (2) Prepare the H-FABP antibody-coated wells as described in “Assay Method 3.(2).” Pipette 100 μ L of the mixed solution prepared in procedure (1) and add to the H-FABP antibody-coated wells using a multichannel pipette.
- (3) Incubate the microtiter wells for 30 min at 15-25°C.
- (4) Wash the microtiter wells three times with wash buffer (300 μ L/well) to remove unbound H-FABP molecules. When a microplate washer is not available, discard the content of each well completely, add 300 μ L of wash buffer to each well and then discard. Repeat this process three times. Remove the residual solution in each well by turning the wells upside down and tapping on a paper towel after the final wash.*
*Note: Never dry the wells completely.
- (5) Add H-FABP antibody-enzyme conjugate (bottle No. 3) (100 μ L) to the microtiter wells, and agitate the wells for 30 sec.
- (6) Incubate the microtiter wells for 30 min at 15-25°C.
- (7) Wash the microtiter wells three times with wash buffer (300 μ L/well) to remove unreacted H-FABP antibody-enzyme conjugate.
- (8) Add a 100 μ L aliquot of substrate solution to each well with a multichannel pipette row by row to assay the HRP enzyme activity.
- (9) Shield the microtiter wells from light with aluminum foil and incubate for 15 min at 15-25°C.
- (10) Terminate the HRP enzyme reaction by addition of 100 μ L of stop solution (bottle No. 5) to each well with a multichannel pipette row by row at the same interval as the addition of substrate solution, and then agitate the wells for 30 sec.
- (11) Measure the absorbance of each well at 492 nm (main wave length) and 620 nm (reference wave length) using a microplate reader within 3 hrs.
- (12) Calculate the concentration of the H-FABP mass in each sample by reference to the standard curve obtained from the seven points of the standard solutions. Express as ng of H-FABP protein/mL of serum or plasma.

5. Preparation of a standard curve and reading of H-FABP concentration in samples

- (1) The graph paper for preparation of a standard curve included in the kit plots the absorbance on the ordinate and the concentration of each standard solution on the abscissa. Plot the absorbance obtained by using each standard solution of

the corresponding H-FABP concentration and draw the best-fit, smooth curve.

- (2) Read from the standard curve the H-FABP concentration corresponding to the absorbance of the sample. The obtained value directly indicates the H-FABP concentration (ng/mL).
- (3) Indicate as “below 1.25 ng/mL” when the obtained value is below the lower limit of detection (1.25 ng/mL). For quantification of high-concentration samples (more than 250 ng/mL), dilute the samples properly with standard solution 0. Then perform the entire procedure and multiply the obtained value by the dilution factor for correction. (Caution: Never dilute the samples with purified water.)



Typical standard calibration curve

■ Cut-off level of H-FABP

A mass concentration of 6.2 ng of H-FABP per mL of serum or plasma is designated as the cut-off value. For clinical assessment this value shows the highest diagnostic efficacy. An H-FABP mass concentration of 6.2 ng/mL of serum or plasma or higher is strongly suggestive of cardiac damage.

■ Clinical assessment

1. Normal range of serum concentration of H-FABP

The range of the serum concentration of the H-FABP mass in normal healthy subjects was investigated by using 75 normal serum samples. The frequency of the H-FABP concentration showed a normal logarithmic distribution. It ranged from less than 1.25 ng/mL to 5.25 ng/mL, and the mean serum H-FABP concentration was 2.75 ng/mL.

The upper limit of the normal reference range of the serum concentration of H-FABP was defined as 5.3 ng/mL.

2. Diagnostic validity

Serum samples from patients with suspected AMI with chest pain, including confirmed AMI and non-AMI, were used for the clinical evaluation of MARKIT-M H-FABP.

(1) Within 3 hrs after the onset of symptoms

Diagnostic sensitivity	89.7%
Diagnostic specificity	72.2%
Diagnostic efficacy	85.5%

(2) Within 6 hrs after the onset of symptoms

Diagnostic sensitivity	93.6%
Diagnostic specificity	69.2%
Diagnostic efficacy	87.8%

(3) Within 12 hrs after the onset of symptoms

Diagnostic sensitivity	92.9%
Diagnostic specificity	67.3%
Diagnostic efficacy	86.2%

3. Non-cardiac cases with high level of H-FABP

Some of serum or plasma samples from patients with aortic dissection, renal failure, regurgitant esophagitis and polymyositis showed a positive level of H-FABP concentration.

■ Instruction in procedure

1. Storage of samples

Seal tubes containing samples with a rubber stopper, etc., if they will not be assayed within 24 hrs, and preserve them at -20°C until use.

2. Freezing-thawing of samples

No influence on the assay result was observed after 5 cycles of freezing-thawing.

3. Storage of standard solutions

Standard solutions can be stored for 1 week at $2-10^{\circ}\text{C}$ or 1 month at -20°C .

■ Interference of co-existing substances

1. Maximum permissible level of interfering substances

Hemoglobin : 470 mg/dL; bilirubin (conjugated) : 22.0 mg/dL; bilirubin (free) : 20.7 mg/dL

2. Anticoagulants

Permissible: Heparin, EDTA, potassium oxalate, sodium citrate

Interfering: Sodium fluoride

3. Sample from patients with autoimmune diseases

Serum and plasma samples containing rheumatoid factor may give falsely high H-FABP concentrations.

■ Cross-reactivity with other FABPs and related cardiac proteins

Mouse anti-human H-FABP monoclonal antibodies were examined for cross-reactivity with other FABPs, such as human L-FABP, porcine H-FABP, bovine H-FABP and canine H-FABP, and various cardiac proteins, such as human skeletal muscle myoglobin and rabbit cardiac muscle myosin light chain. The degree of cross-reactions with these proteins was less than 0.1%.

■ Performance

1. Sensitivity

The measured absorbance “A” of standard solutions 0, 5, 10, 25, 50, 100 and 250 should be as follows:

- (1) $A(250) - A(0)$ is more than 1.0.
- (2) $A(0) < A(5) < A(10) < A(25) < A(50) < A(100) < A(250)$
- (3) Absorbance of standard solution 2.5 prepared using standard solutions 0 and 5: $A(2.5)$ should be larger than $A(0)$ and smaller than $A(5)$.

2. Specificity

Control plasma (H-FABP, 50-200 ng/mL) should show a value within 85-115% of its known concentration with this kit.

3. Reproducibility

When two distinct samples (H-FABP, 50-200 ng/mL) are determined 10 times simultaneously, the coefficient of variation in their absorbance should be less than 10%.

■ Range of standard curve

H-FABP 0-250 ng/mL

■ Precautions for use or handling

1. General Precautions

- (1) Do not use the kit for any purpose not mentioned in this manual.
- (2) Be sure to use reagents, including standard solutions, H-FABP antibody-coated

wells, H-FABP antibody-enzyme conjugate, substrate tablets and substrate diluent buffer, of the same lot. Do not use in combination with any reagents from different lots.

- (3) If the reagents of this kit, especially the stop solution, get into the eyes or mouth, applying appropriate first aid, such as thorough washing, and then consult a doctor if necessary.
- (4) Do not use reagents after their expiration date.
- (5) Do not freeze the H-FABP antibody-coated wells or the H-FABP antibody-enzyme conjugate.
- (6) Before using the kit, be sure to read the operating manuals for the equipment required for this assay.
- (7) Use this assay kit as soon as possible once the package is opened. For storage, cap/seal all the reagents and keep them in a cool, dark place (2-10°C).
- (8) The standard assay is recommended to be performed in duplicate.
- (9) All reagents should be added in the exact order stated in the procedures. Samples and standard solutions should be treated under the same conditions.

2. Avoiding hazards to the user

(1) Viruses

The human H-FABP used as the standard in this kit is negative for HBs antigen, HIV antibody and HCV antibody.

(2) Pipetting

Never use your mouth to pipette the reagents or samples at any time. Never fail to use a pipette with a disposable tip.

3. Handling of wastes

Inactivate any viruses in samples, reagents and used apparatuses when the test is completed by the following methods.

- (1) Autoclave (132°C, 1 hr).
- (2) Submerge in 1-5% sodium hypochlorite solution at room temperature for 2 hrs.
- (3) Submerge in 1 mol/L sodium hydroxide solution at room temperature for 1 hr.
- (4) Submerge in 3% sodium dodecyl sulfate solution at 100°C for 5 min.

■ Storage method and expiry period

Storage : Store in a cool place (2-10°C), protected from light. Avoid freezing.

Expiry period: 2 years

■ Package units

MARKIT-M H-FABP: 1 kit (96 tests)

■ References

- 1) Ockner, R.K., et al.: Historic overview of studies on fatty acid-binding proteins. *Mol. Cell. Biochem.* 98: 3-9, 1990.
- 2) Kaikaus, R.M., et al.: Functions of fatty acid-binding proteins. *Experientia* 46: 617-630, 1990.
- 3) Ohkaru, Y., et al.: Development of a sandwich enzyme-linked immunosorbent assay for the determination of human heart type fatty acid-binding protein in plasma and urine by using two different monoclonal antibodies specific for human heart fatty acid-binding protein. *J. Immunol. Methods* 178: 99-111, 1995.
- 4) Tsuji, R., et al.: Human heart-type cytoplasmic fatty acid-binding protein in serum and urine during hyperacute myocardial infarction. *Int. J. Cardiol.* 41: 209-217, 1993.
- 5) Sohmiya, K., et al.: Plasma and urinary heart-type cytoplasmic fatty acid-binding protein in coronary occlusion and reperfusion induced myocardial injury model. *J. Mol. Cell. Cardiol.* 25: 1413-1426, 1993.
- 6) Tanaka, T., et al.: Serum and urinary human heart fatty acid-binding protein in acute myocardial infarction. *Clin. Biochem.* 24: 195-201, 1991.
- 9) Paulussen, R.J.A., et al.: Immunochemical quantitation of fatty acid-binding proteins. Tissue distribution of liver and heart FABP types in human and porcine tissues. *Int. J. Biochem.* 22: 393-398, 1990.
- 10) Adams, J.E. III, et al.: Biochemical markers of myocardial injury, Is MB creatine kinase the choice for the 1990s? *Circulation* 88: 750-763, 1993.
- 11) Pauleo, P.R. and Roberts, R.: An update on cardiac enzymes. *Cardiol. Clin.* 6: 97-109, 1988.
- 12) Veerkamp, J.H., et al.: Cytoplasmic fatty acid-binding proteins: their structure and genes. *Prog. Lipid Res.* 34: 17-52, 1995.
- 13) Glatz, J.F.C., et al.: Fatty-acid-binding protein as a plasma marker for the estimation of myocardial infarct size in humans. *Br. Heart J.* 71: 135-140, 1994.
- 14) Wodzig, K.W.H., et al.: Estimation of myocardial infarct size from plasma myoglobin or fatty acid-binding protein. Influence of renal function. *Eur. J. Clin. Chem. Clin. Biochem.* 35: 191-198, 1997.

■ **Exporter**

SCETI K.K.

DF Kasumigaseki Place 3-6-7 Kasumigaseki

Chiyoda-ku, Tokyo 100-0013 JAPAN

TEL: +(81)3-5510-2347

FAX: +(81)3-5510-0134

e-mail: exp-pet@sceti.co.jp

URL: <http://www.sceti.co.jp/export/>

■ **Manufacturer**

DS Pharma Biomedical Co., Ltd