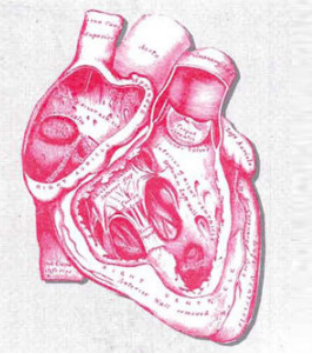
Advanced ImmunoChemical Inc.
Providing for the Advancement of Medical Science

ASSAY NOTES
Product Information

Courtesy of HyTest, Ltd

Heart Type Fatty Acid Binding Protein (H-FABP)



Fatty acid-binding proteins (FABPs) are a group of small (12-15 kDa) cytoplasmic proteins that are abundant in tissues with active fatty acid metabolism. They participate in the intracellular transportation of long-chain fatty acids. The heart-type FABP (H-FABP) is composed of 132 amino acids and it is one of the most abundant proteins in the myocardial tissue that con- stitutes 5-15% of the cytoplasmic proteins in the human heart.

H-FABP in diagnostics

H-FABP is one of the early markers of acute coronary syndrome. Its specificity is much higher than that of myoglobin, which is another early marker of cardiac injury (1). Following an acute cardiac event, the level of H-FABP rapidly increases in the blood. It can be detected much earlier than cardiac troponins, which are the most specific biomarkers of myocardial infarction. H-FABP concentration peaks within six hours and returns back to normal after 12-24 hours (see Figure 1). Following this it loses its clinical value.

H-FABP can provide valuable information to support the diagnosis of patients suspected of having an acute cardiac event. H-FABP alone is not sufficiently specific as a marker for cardiac injuries since it is also expressed in other tissues besides the heart. Furthermore, its diagnostic window is not as wide as that of troponins, for example. However, as part of a multi-marker panel, H-FABP brings additional value in terms of supporting clinical diagnostics decisions. It is applied in emergency triage of patients with acute coronary syndromes (2). H-FABP measurements could also be used to identify patients with a low risk of acute myocardial infarction (AMI) and consequently accelerate their discharge from hospital (3).

CLINICAL UTILITY

- Early marker of acute cardiac syndrome
- Prediction of adverse prognosis of pulmonary embolism

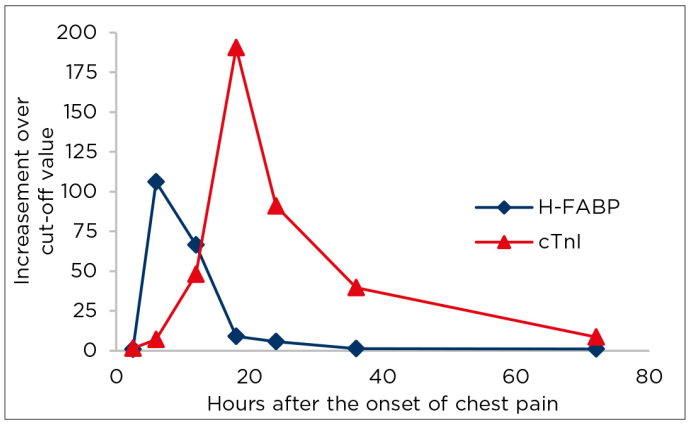


Figure 1. Serial measurements of H-FABP and cardiac troponin I concentrations in the blood of a representative AMI patient. *The concentrations of two cardiac biomarkers were determined at seven different time points after the onset of chest pain as indicated in the picture. Concentration profiles show that H-FABP peaks earlier than cardiac troponin I. Its level in blood also normalizes sooner.*

H-FABP has also been identified as a biomarker that is useful in regard to the prediction of adverse prognosis in patients with pulmonary embolism. European Society of Cardiology guidelines on the diagnosis and management of acute pulmonary embolism (4) list H-FABP as one of the biomarkers that could be used for risk stratification in confirmed pulmonary embolism cases.

Reagents for H-FABP immunoassay

We offer eight different monoclonal antibodies that are specific to H-FABP. They allow the development of sensitive immunoassays with a detection limit of 0.05 g/L. In addition, we also offer native human FABP that is purified from cardiac tissue and FABP- free serum.

Monoclonal antibodies specific to H-FABP

Sandwich immunoassay for the quantitative detection of H-FABP

Calibration curves for several two-site combinations are shown in Figure 2. The best antibody combinations for sandwich immunoassays are set out in Table 1.

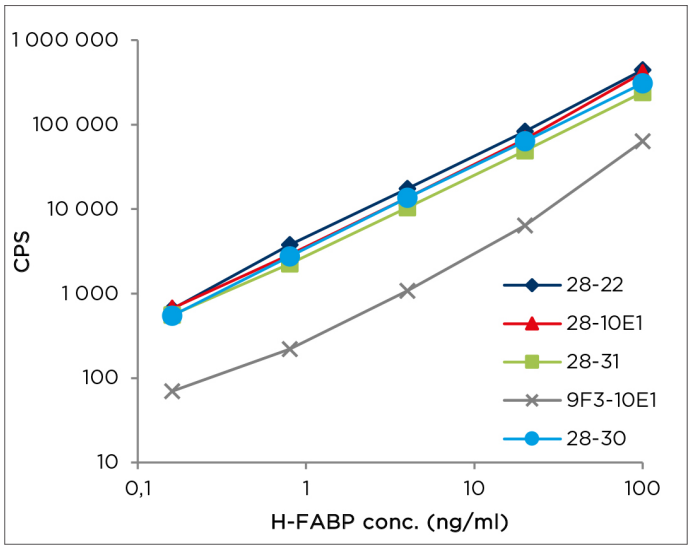


Figure 2. Calibration curves for H-FABP sandwich fluoroimmunoassays. *Detection antibodies were labeled with a stable Eu3+ chelate. The antigen that was used was purified native H-FABP.*

Table 1. The best MAb combinations for the development of a quantitative H-FABP sandwich immunoassays. *Data is based on the results obtained using time-resolved fluorescence im- munoassay.*

Capture	Detection
28	22
28	10E1
28	31
9F3	10E1

Measuring H-FABP performance in AMI clinical samples

Figure 3 shows the results that were obtained with several different MAb combinations from the serum of six AMI patients.

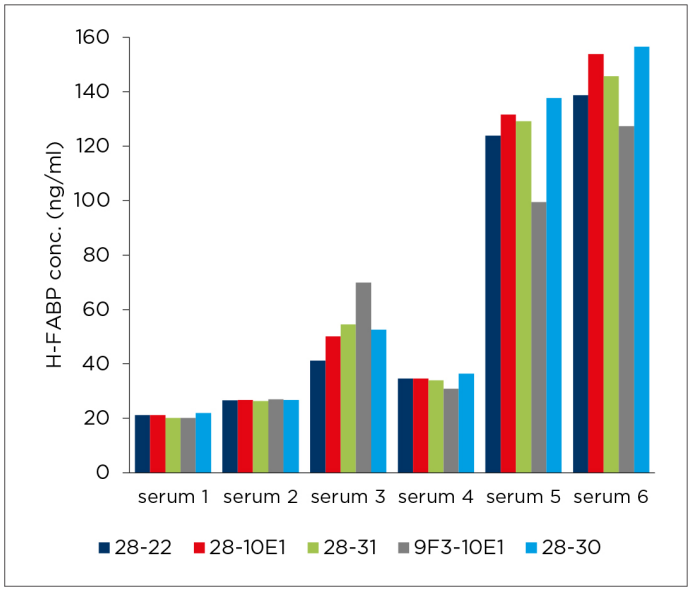


Figure 3. H-FABP concentration in serum samples of six different AMI patients measured using several antibody combinations. *All of the tested immunoassays gave highly comparable results.*

H-FABP immunodetection in direct ELISA

All of the anti-H-FABP MAbs recognize human FABP in direct ELISA (see Figure 4).

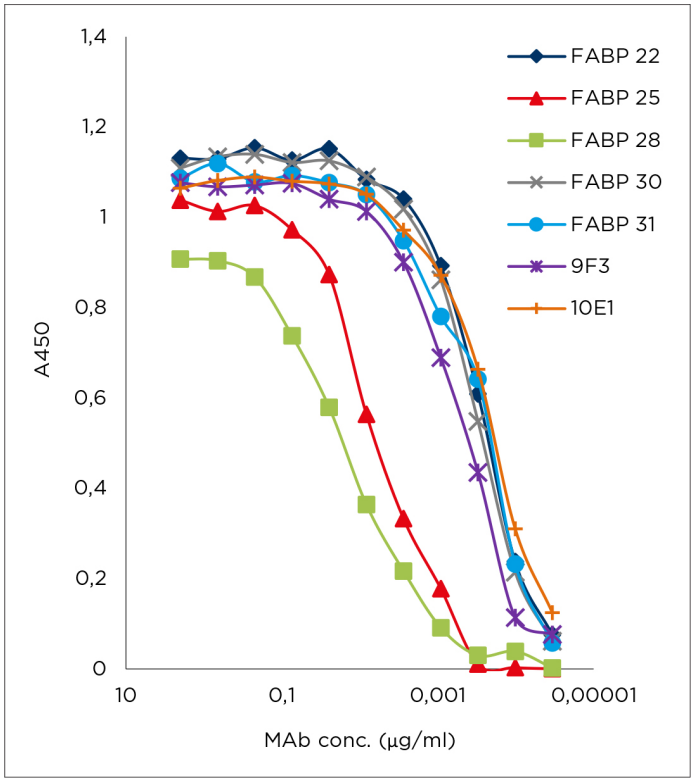


Figure 4. Detection of purified native H-FABP in direct ELISA. *40 ng of human H-FABP was coated onto microtiter plate wells and titrated with anti-H-FABP MAbs indicated in the figure. MAbs 25 and 28 displayed a lower response than others. This is most likely caused by a change in the structure or visibility of their specific epitopes on H-FABP following the coating of the antigen on the plate surface.*

H-FABP immunodetection in Western blotting

All anti-H-FABP MAbs were tested for their ability to detect human H-FABP in Western blotting. For this application, using MAbs 22, 30 or 31 is recommended (see Figure 5).

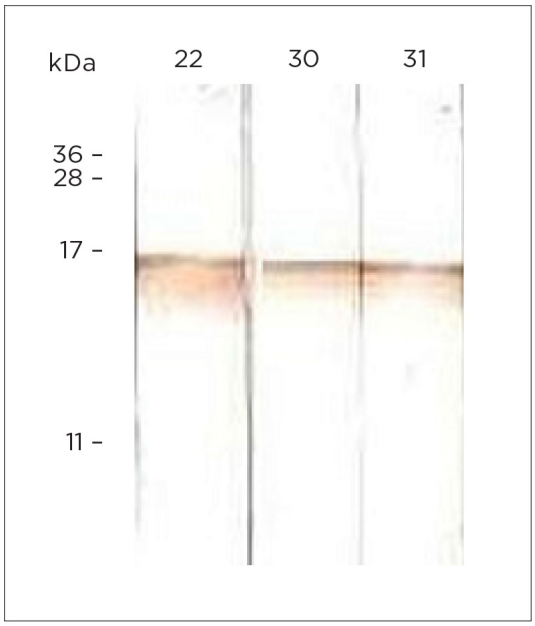


Figure 5. Immunodetection of H-FABP by MAbs 22, 30 and 31 in Western blotting after PAGE in reducing conditions. *Secondary antibody (anti-mouse IgG) was conjugated with HRP.*

Additional reagents

Native H-FABP

Native FABP was purified from human cardiac tissue with several chromatographic procedures. Its purity is >95% as determined by densitography following SDS-gel electrophoresis in reducing conditions (data not shown). In SDS-PAGE it migrates as a single band with an apparent molecular weight of 15 kDa.

FABP-free serum

FABP-free serum was prepared from pooled normal human serum by immunoaffinity chromatography. The matrix for affinity sorbent utilizes three monoclonal antibodies with different epitope specificity. FABP -free serum contains a maximum 0.5 ng/ml of H-FABP as determined by ELISA. It can be used as a matrix for standard and calibrator preparations.

References

1. Wu A. Cardiac markers. Second edition, 2003, Humana Press

2. Alhadi HA and Fox KA. Do we need additional markers of myocyte necrosis: the potential value of heart fatty-acid-binding protein, QJM. 2004, 97 (4): 187–198.

3. Young JM. et al. Heart fatty acid binding protein and cardiac troponin: development of an optimal rule-out strategy for acute myocardial infarction, BMC Emerg Med. 2006, 16: 34.

4. Konstantinides SV et al.; Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC). 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. Eur Heart J. 2014, 35 (43): 3033-3069.

Ordering Information:

MONOCLONAL ANTIBODIES

Product Name	Cat #	MAb	Subclass	Remarks
Fatty acid binding protein	2-FABP	5B5	IgG1	EIA
		9F3	IgG1	EIA
		10E1	IgG1	EIA
		22	IgG1	EIA, WB
		25	IgG1	EIA
		28	IgG1	EIA
		30	IgG1	EIA, WB
		31	IgG1	EIA, WB

ANTIGENS

Product Name	Cat. #	Purity	Source
Fatty acid binding protein	8-FABP	>95%	Human cardiac muscle

DEPLETED SERUM

Product Name	Cat. #	Source
Fatty acid binding protein free serum	11-FA-fs	Pooled normal human serum

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