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 constitutes 5-15% of the cytoplasmic proteins in the human heart.

CLINICAL UTILITY

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

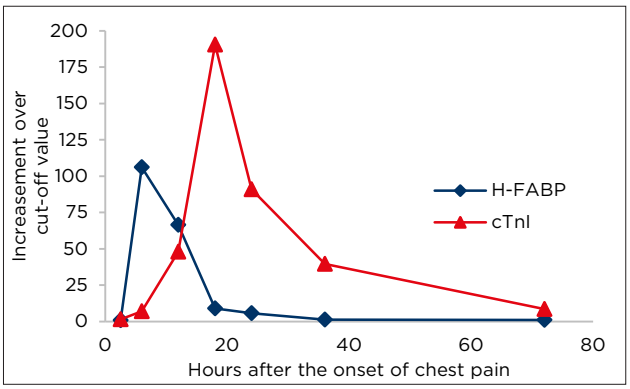

Figure 1. Serial measurements of H-FABP and cardiac troponin I concentrations in the blood of a representative AMI patient. The concentrations of two cardiac biomarkers were determined at seven different time points after the onset of chest pain as indicated in the picture. Concentration profiles show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

H-FABP 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).

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 provide 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 our in-house fluoroimmunoassay platform). In addition, we also provide 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

In order to select the best performing antibody combinations, we tested all of the MAbs in pairs as capture and detection antibodies. 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.

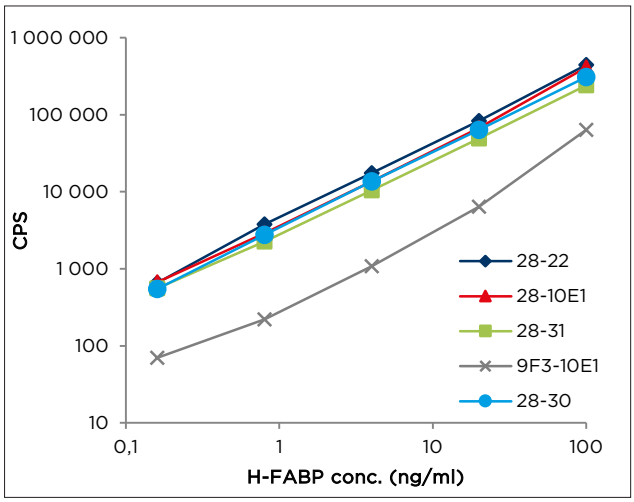

Figure 2. Calibration curves for H-FABP sandwich fluoroimmunoassays. Detection antibodies were labeled with a stable Eu^{3+} chelate. The antigen that was used was purified native H-FABP.

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

Capture	Detection
28cc	22
28cc	10E1
28cc	31
9F3cc	10E1

Measuring H-FABP in patient samples

In order to confirm the performance of the antibodies with clinical samples, we have determined the concentration of H-FABP in serum samples from hundreds of patients who have been diagnosed with acute myocardial infarction. Figure 3 shows the results that were obtained with several different MAb combinations from the serum of six AMI patients.

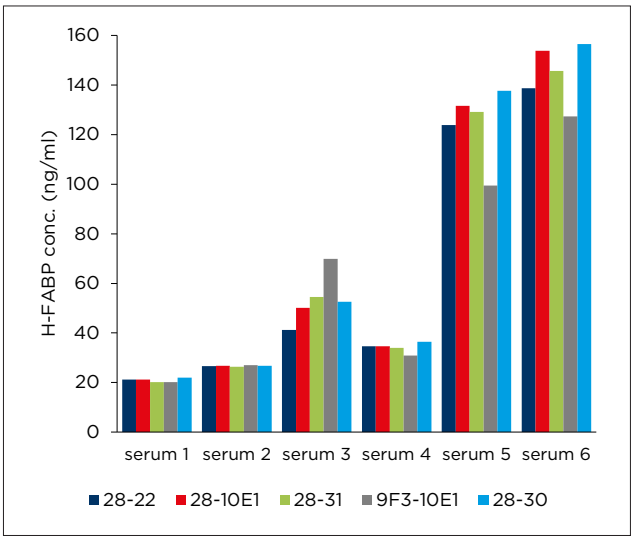

Figure 3. H-FABP concentration in serum samples of six different AMI patients measured using several antibody combinations. All of the tested immunoassays gave highly comparable results.

H-FABP immunodetection in direct ELISA

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

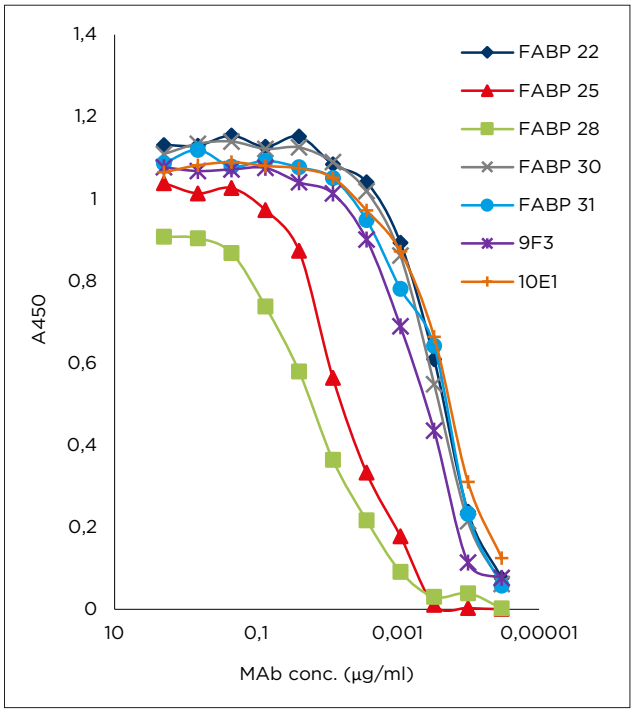

Figure 4. Detection of purified native H-FABP in direct ELISA. 40 ng of human H-FABP was coated onto microtiter plate wells and titrated with anti-H-FABP MAbs indicated in the figure. MAbs 25 and 28 displayed a lower response than others. This is most likely caused by a change in the structure or visibility 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, we recommend using MAbs 22, 30 or 31 (see Figure 5).

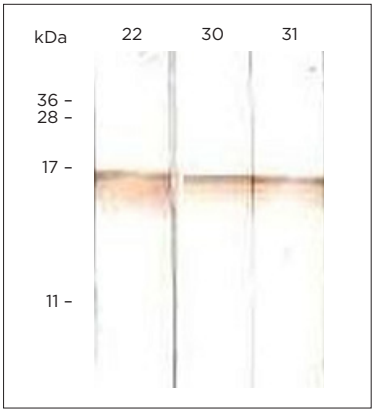

Figure 5. Immunodetection of H-FABP by MAbs 22, 30 and 31 in Western blotting after PAGE in reducing conditions. Secondary antibody (anti-mouse IgG) was conjugated with HRP.

Native H-FABP

Native FABP is purified from human cardiac tissue with several chromatographic procedures. Its purity is >95% as determined by densitography following SDS-gel electrophoresis in reducing conditions (see Figure 6). In SDS-PAGE it migrates as a single band with an apparent molecular weight of 15 kDa.

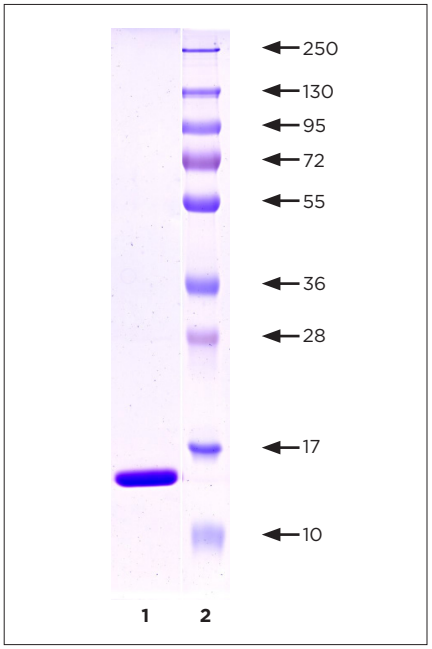

Figure 6. SDS-PAGE of purified H-FABP (Cat.# 8F65) in reducing conditions. The gel was stained using Coomassie Brilliant Blue R-250.
Lane 1: H-FABP, 2 µg
Lane 2: MW standard

FABP-free serum

FABP-free serum is 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.

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Fatty acid binding protein	4F29	5B5	IgG1	EIA
		9F3cc	IgG1	*In vitro*, EIA
		10E1	IgG1	EIA
		22	IgG1	EIA, WB
		25	IgG1	EIA
		28cc	IgG1	*In vitro*, EIA
		30	IgG1	EIA, WB
		31	IgG1	EIA, WB

ANTIGEN

Product name	Cat. #	Purity	Source
Fatty acid binding protein	8F65	>95%	Human cardiac muscle

DEPLETED SERUM

Product name	Cat. #	Source
Fatty acid binding protein free serum	8FFS	Pooled normal human serum

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced in vivo. MAbs produced in vitro are expected to have similar performance.

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.

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