MSD® MULTI-SPOT Assay System

Human FABP3 Kit

1-Plate Kit	K151HTD-1
5-Plate Kit	K151HTD-2
25-Plate Kit	K151HTD-4

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MSD Biomarker Assays

Human FABP3 Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Ordering Information

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Introduction

Fatty acid-binding proteins (FABPs) are members of a superfamily of lipid-binding proteins that facilitate fatty acid transport, cell growth and differentiation, cellular signaling, gene transcription, cyto-protection, and programmed cell death.[1] FABP3, or heart-type cytoplasmic FABP (hFABP), is a 14.5 kDa protein that facilitates intracellular transport of long-chain fatty acids (LCFA) into muscle cells. By non-covalent binding, FABP3 increases LCFA concentration in the aqueous cytoplasm and facilitates diffusion from membranes to mitochondria for oxidation.[2] FABP3 also increases myocyte glucose uptake through AMPK activation.[3] FABP3-facilitated lipid accumulation and unregulated glucose uptake may contribute to fat deposition in myocytes and concomitant insulin resistance and apoptosis. Elevated levels of circulating FABP3 have been closely associated with acute coronary syndrome, acute myocardial damage, cardiac abnormalities, stroke, and obstructive sleep disorder.[4,5] FABP3 may also serve as a prognostic indicator of myocardial infarction.[6]

Principle of the Assay

MSD biomarker assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human FABP3 is a sandwich immunoassay (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into a SECTOR® Imager where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

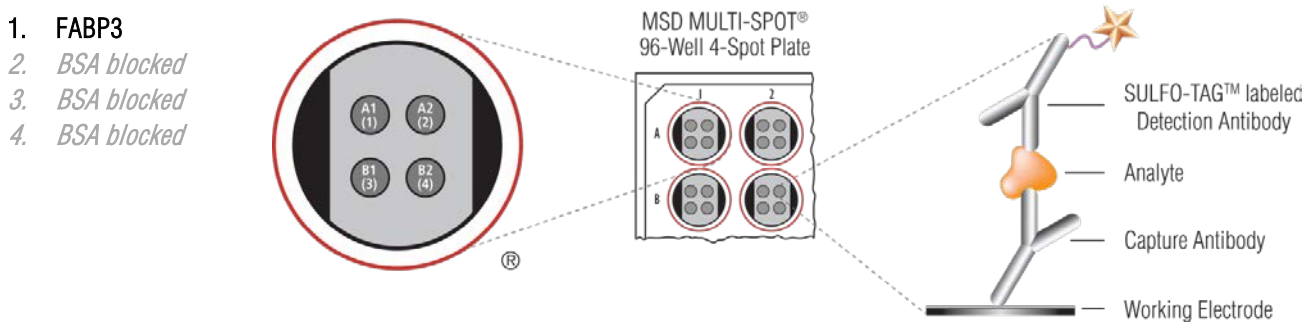


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K151HTD-1	K151HTD-2	K151HTD-4
MULTI-SPOT 96-Well, 4-Spot Human FABP3 Plate N451HTA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu FABP3 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human FABP3 Calibrator (20X)	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 µL ea)
Diluent 12 R50JA-4 (10 mL), R50JA-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Diluent 3 R50BA-4 (5 mL), R50BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL)	5 bottles (25 mL ea)
Blocker A Kit (Blocker A [dry] in 250 mL bottle and 50 mL bottle of 5X Phosphate Buffer) R93AA-2 (250 mL)	RT	1 kit (250 mL)	1 kit (250 mL)	5 kits (250 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Required Material and Equipment (not supplied)

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Microcentrifuge tubes for preparing serial dilutions
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Deionized water

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional safety information is available in the product Material Safety Data Sheet, which can be obtained from MSD Customer Service.

[1] SULFO-TAG–conjugated detection antibodies should be stored in the dark.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate Diluent 3 and Diluent 12 into aliquots appropriate for the size of your needs before refreezing.

Prepare Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Standards

MSD supplies calibrator for the Human FABP3 Kit at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 3-fold serial dilution steps and a zero calibrator blank. Signals from the blank should be excluded when generating the curve. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions.

Standard	Human FABP3 (ng/mL)	Dilution Factor
Stock Calibrator	2000	
STD-01	100.0	20
STD-02	33.3	3
STD-03	11.1	3
STD-04	3.70	3
STD-05	1.23	3
STD-06	0.412	3
STD-07	0.137	3
STD-08	0	n/a

To prepare 7 standard solutions plus a zero calibrator blank for up to 3 replicates:

1) Prepare the highest standard by adding 15 µL of stock calibrator to 285 µL of Diluent 12. Mix well.
2) Prepare the next standard by transferring 100 µL of the highest standard to 200 µL of Diluent 12. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 12 as the blank.

Dilute Samples

For human serum and plasma samples, MSD recommends 2-fold dilution in Diluent 12; However, you may need to adjust the dilution factor for the sample set under investigation. To dilute sample 2-fold, add 75 µL of sample to 75 µL of Diluent 12.

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working detection antibody solution is 1X.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu FABP3 Antibody
- ❑ 2940 µL of Diluent 3

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

Notes

1. **Add Blocker A Solution:** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   Shaking the plate typically accelerates capture at the working electrode.

2. **Wash and Add Sample:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 50 µL of sample (standards, controls, or unknowns) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
   You may prepare detection antibody solution during incubation.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
   You may prepare diluted read buffer during incubation.

   You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

4. **Wash and Read:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required before reading the plate.

   Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

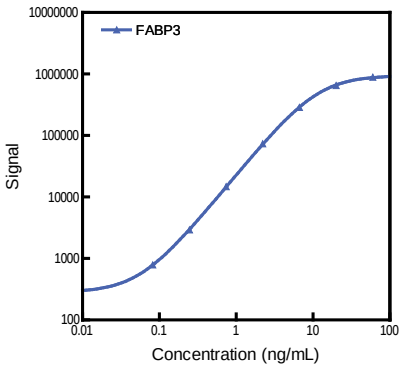
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate the standard curve that will be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) that allows accurate quantification without the need for dilution in many cases. By default, the software uses a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Typical Data

The following standard curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of the standards.



FABP3		
Conc. (ng/mL)	Average Signal	%CV
0	356	6.0
0.137	785	3.6
0.412	2919	1.6
1.23	14 727	3.6
3.70	73 253	3.9
11.1	286 538	3.0
33.3	648 383	2.5
100.0	890 584	2.8

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank). The LLOD shown below was calculated based on 4 runs.

	FABP3
Average LLOD (ng/mL)	0.103
LLOD Range (ng/mL)	0.0952–0.107

Assay Components

Calibrator

The assay calibrator uses recombinant FABP3 protein, residues 1–133, expressed in *E. coli*.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
FABP3	Mouse monoclonal	Goat Polyclonal

References

1. Zimmerman AW, Veerkamp JH. New insights into the structure and function of fatty acid-binding proteins. Cell Mol Life Sci. 2002 Jul;59(7):1096-116.
2. Glatz JF, et al. Cytoplasmic fatty acid-binding protein facilitates fatty acid utilization by skeletal muscle. Acta Physiol Scand. 2003 Aug;178(4):367-71.
3. Kusudo T, et al. Fatty acid binding protein 3 stimulates glucose uptake by facilitating AS160 phosphorylation in mouse muscle cells. Genes Cells. 2011 Jun;16(6):681-91.
4. Petzold T, et al. Heart-type fatty acid binding protein (hFABP) in the diagnosis of myocardial damage in coronary artery bypass grafting. Eur J Cardiothorac Surg. 2001 Jun;19(6):859-64.
5. Kilcullen N, et al. Heart-type fatty acid-binding protein predicts long-term mortality after acute coronary syndrome and identifies high-risk patients across the range of troponin values. J Am Coll Cardiol. 2007 Nov 20;50(21):2061-7.
6. Karbek B, et al. Heart-type fatty acid binding protein (H-FABP): relationship with arterial intima-media thickness and role as diagnostic marker for atherosclerosis in patients with ımpaired glucose metabolism. Cardiovasc Diabetol. 2011 May 2;10:37.

Summary Protocol

Human FABP3 Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human FABP3 assay.

Sample and Reagent Preparation

Bring all reagents to room temperature and thaw the calibrator on ice.

Prepare Blocker A solution.

Prepare standard solutions using the supplied calibrator:

- Dilute the stock calibrator 20-fold in Diluent 12.
- Perform a series of 3-fold dilution steps and prepare a zero calibrator blank.

Dilute samples 2-fold in Diluent 12 before adding to the plate.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 3.

Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-fold with deionized water.

Step 1: Add Blocker A Solution

Add 150 µL/well of Blocker A solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 2: Wash and Add Sample

Wash plate 3 times with 300 µL/well of PBS-T.

Add 50 µL/well of sample (standards, controls, or unknowns).

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with 300 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 4: Wash and Read Plate

Wash plate 3 times with 300 µL/well of PBS-T.

Add 150 µL/well of 2X Read Buffer T.

Analyze plate on SECTOR Imager.

Plate Diagrams