Human Fatty-acid binding protein 4 immunoassay kit

Catalogue Number: 31030

For the quantitative determination of human FABP4 concentrations
in serum, plasma and cell culture supernate samples.

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

Version: 1.0 **FOR RESEARCH USE ONLY**

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INTRODUCTION

Fatty-acid binding protein 4 (FABP4), also termed adipocyte fatty-acid binding protein (A-FABP), or aP2, is a novel adipocyte-expressed factor which accounted for ~6% of total cellular proteins. Several animal experiments suggested that FABP-4 plays a key role in the link between obesity and various features of metabolic syndrome[1]. Mice with targeted disruption of FABP-4 accompany FABP-5 almost completely protect against diet-induced obesity, insulin resistance, dyslipidemia, type 2 diabetes, and fatty liver disease[2]. Studies in human found FABP-4 serum levels were significantly increased in overweight and obese subjects, which predicted the risk to develop a metabolic syndrome and type 2 diabetes[3,4]. Additionally, serum FABP-4 levels were associated with nonalcoholic fatty liver disease, carotid atherosclerosis and coronary artery disease[5-7].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich ELISA. The immunoplate is pre-coated with a mouse monoclonal antibody specific for human FABP4. Standards and samples are pipetted into the wells and any Human FABP4 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin labelled polyclonal antibody specific for human FABP4 is added to the wells. After wash step to remove any unbound reagents, streptavidin-HRP conjugate is added, after the last wash step, an HRP substrate solution is added and colour develops in proportion to the amount of human FABP4 bound initially. The assay is stopped and the optical density of the wells determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human FABP4, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Human FABP4 ELISA kit is designed for quantification of Human FABP4 in serum, plasma, and adipocyte extracts or cell culture media samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Micro-titre Strips (96 wells)-Coated with a mouse monoclonal antibody against human FABP4.
2. 10×Wash Buffer-50 ml.
3. 5×Assay Buffer-20 ml.
4. 100×Detection Antibody-A biotin labelled polyclonal antibody against human FABP4, 0.12 ml.
5. Human FABP4 Standard-25 ng of recombinant human FABP4 in a buffered protein base, lyophilised.
6. 200×STP-HRP solution-0.06 ml.
7. Substrate solution- 12 ml, ready for use.
8. Stop Solution-12 ml, ready for use.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. 96-well plate or manual strip washer.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the Human FABP4 microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored for up to one month at 2-8°C.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer.

Prepare 1×Assay buffer by mixing the 5×Assay Buffer (20 ml) with 80 ml of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash Buffer (50 ml) with 450 ml of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution.

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 μl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection Antibody to 2-8°C immediately after the necessary volume is removed.

D. 1× STP-HRP solution.

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 μl of the 1× STP-HRP solution is required per well. Prepare only as much 1×STP-HRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed**.**

PREPARATION OF STANDRADS AND SAMPLES

Human FABP4 Standards: Reconstitute the lyophilised standard with 1 ml of 1×Assay buffer to generate a standard stock solution of 25 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1×Assay buffer	Concentration
25.0 ng/ml stock	-	25.0 ng/ml
250 µl of 25.0 ng/ml stock	250 µl	12.5 ng/ml
250 µl of 12.5 ng/ml std.	250 µl	6.25 ng/ml
250 µl of 6.25 ng/ml std.	250 µl	3.12 ng/ml
250 µl of 3.12 ng/ml std.	250 µl	1.56 ng/ml
250 µl of 1.55 ng/ml std.	250 µl	0.78 ng/ml
250 µl of 1.55 ng/ml std.	250 µl	0.39 ng/ml

1×Assay buffer serves as the zero standard (0 ng/ml). The reconstituted standard stock should be aliquoted and frozen at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample preparation

Serum or plasma sample is generally required a 3-fold dilution in this assay. A suggested dilution step is to add 100 µl of sample to 200 µl of 1×Assay buffer. Cellular extract and culture media dilutions will vary and need to be optimized by the user, also use 1×Assay buffer to prepare these samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µl of standards and samples to each well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µl of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 3 times as in step 2.
5. Add 100 µl of 1×STP-HRP solution to each well, incubate at room temperature for 20 minutes.
6. Wash each well 4 times as described in step 2.
7. Add 100 µl of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 µl of Stop solution to each well, mix well by gently tapping the plate.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

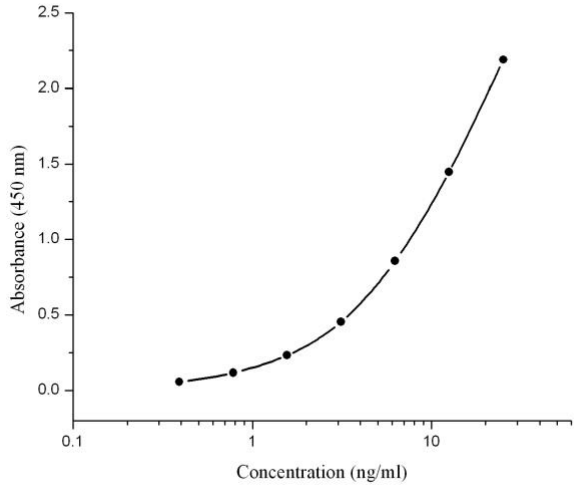
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against FABP4 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine FABP4 concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

FABP4 (ng/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.1	0
0.39	0.158	0.058
0.78	0.228	0.118
1.56	0.332	0.232
3.12	0.554	0.454
6.25	0.957	0.857
12.5	1.547	1.447
25	2.289	2.189

Human FABP4 standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity: The lowest level of FABP4 that can be detected by this assay is 0.39 ng/ml.

B. Specificity: The antibodies used in this assay are specific to human FABP4 and do not cross-react with mouse and rat FABP4, and other cytokine or hormone molecules.

C. Precision:

Intra-assay Precision (Precision within an assay) C.V.< 4.1%.
Inter-assay Precision (Precision between assays) C.V.< 4.5%.

REFERENCES:

[1] Makowski L, et al. (2004) *J Nutr*. 134: 2464S–2468S.
[2] Maeda K, et al. (2005) *Cell Metab.* 1: 107–119.
[3] Xu A, et al. (2006). *Clin Chem*. 52(3):405-13.
[4] Xu A, et al. (2007). *Circulation.* 115:1537–1543.
[5] Rhee EJ, et al. (2009) *Eur J Endocrinol.* 160(2):165-72.
[6] Tso AW, et al. (2007) *Diabetes Care*. 30(10):2667-72
[7] J. Hyun Koh, et al. (2009) *Diabetes Care.* 32(1): 147 - 152.

SUMMARY OF ASSAY PROCEDURE

Add 100 µl of Standard or sample to each well.
↓
Incubate at room temperature for 1 hour.
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Wash each well three times.
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Add 100 µl of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Wash each well three times.
↓
Add 100 µl of 1×STP-HRP solution to each well.
↓
Incubate at room temperature for 20 minutes.
↓
Wash each well four times.
↓
Add 100 µl of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µl of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
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Calculation