ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Mouse Fatty Acid Binding Protein 4 (FABP4) ELISA Kit

(Catalog Number: 32030)

For the quantitative determination of mouse FABP4 concentrations in serum, plasma or cell culture supernate samples

IMD (Hong Kong)
Address: Unit 513, 5/F, Biotech Centre 2, No. 11 Science Park West Avenue, Hong Kong Science Park, Sha Tin, Hong Kong
Website: www.immunodiagnostics.com.hk
Email: info@immunodiagnostics.com.hk
Tel: (+852) 3502 2780

IMD (Canada)
Address: 3330 Bayview Avenue, Block #6, Toronto, M2M 3R8, Ontario, Canada
Email: info@immunodiagnostics.ca
Tel: +1-437-886-5136

Version 23/01

TABLE OF CONTENTS

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 FABP4 plays a key role in the link between obesity and various features of metabolic syndrome[1]. Mice with targeted disruption of FABP4 accompany FABP5 almost completely to protect against diet-induced obesity, insulin resistance, dyslipidemia, type 2 diabetes, and fatty liver disease[2]. Studies in human found FABP4 serum levels were significantly increased in overweight and obese subjects, which predicted the risk to develop metabolic syndrome and type 2 diabetes[3,4]. Additionally, serum FABP4 levels were associated with non-alcoholic fatty liver disease, carotid atherosclerosis and coronary artery disease[5-7].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter plate is pre-coated with affinity purified mouse monoclonal antibody against mouse FABP4. Standards and samples are pipetted into the wells and any mouse FABP4 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-labelled polyclonal antibody against mouse FABP4 is added to the wells. After wash step to remove any unbound reagents, streptavidin-horseradish peroxidase (STP-HRP) conjugate is added, after the last wash step, an HRP substrate solution 3,3′,5,5′-Tetramethylbenzidine (TMB) is added and color develops in proportion to the amount of mouse FABP4 bound initially. Color reaction is stopped by 2M H_2SO_4 and the optical density of the wells are determined using a microtiter plate reader at 450nm. Since the increases in absorbance are directly proportional to the amount of captured mouse FABP4, the unknown sample concentration can be calculated from the standard curve included in each assay.

INTENDED USE

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

REAGENTS SUPPLIED

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

1. Microtiter Strips (96 wells), coated with a mouse monoclonal antibody against mouse FABP4
2. 10×Wash Buffer, 50 mL
3. 5×Assay Buffer, 20 mL
4. 100×Detection Antibody, a biotin labelled polyclonal antibody against mouse FABP4, 0.12 mL
5. Mouse FABP4 Standard, 25 ng of recombinant mouse FABP4, lyophilized
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 mouse FABP4 microtiter plate, 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.

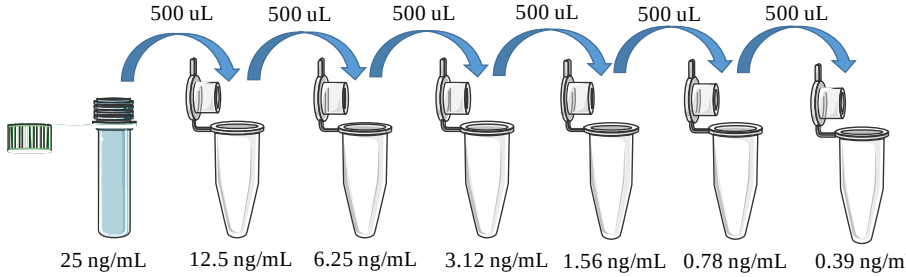
D. 1× STP-HRP solution

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 STANDARDS AND SAMPLES

Mouse FABP4 Standards: Reconstitute the lyophilized 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.

Pipette 500 µL of 1×Assay buffer to 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 ng/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



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 generally requires a **200-fold** dilution in this assay. A suggested dilution step is to add 1 µL of sample to 199 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. It is recommended that the users establish their own dilution factors based on the concentration range of their 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.

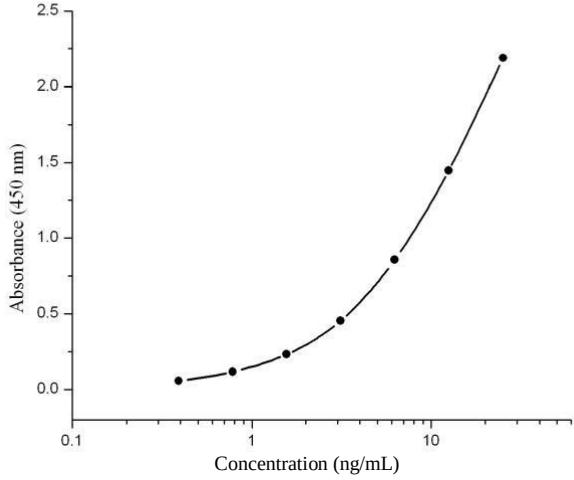
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against FABP4 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine FABP4 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.

Mouse FABP4 (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.110	0
0.39	0.168	0.058
0.78	0.227	0.117
1.56	0.365	0.255
3.12	0.615	0.505
6.25	0.922	0.812
12.5	1.451	1.341
25	2.128	2.018

Mouse 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 mouse FABP4 and do not cross-react with 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.
↓
Wash each well three times.
↓
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.
↓
Calculation