AFABP (human)

ELISA KIT

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AFABP (human)

Enzyme Immunoassay kit

#A05181.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed
& validated by Bertin Bioreagent

Fabriqué en France
Made in France

#A11181
Version: 0122

Table of contents

96 wells

Storage: +4°C

Expiry date: stated on the package

This kit contains:

Designation	Item #	Quantity per kit	Form
AFABP (human) precoated Microtiter Plate	A08181	1	Ready to use
Streptavidin-HRP Tracer	A22010	1	Liquid
AFABP (human) concentrated Biotin-labelled Antibody (100x)	A40181	1	Liquid
AFABP (human) Standard	A06181	1	Lyophilised
AFABP (human) Quality Control LOW	A010181_L	1	Lyophilised
AFABP (human) Quality Control HIGH	A010181_H	1	Lyophilised
Biotin-labelled Antibody Dilution Buffer	A07011	1	Lyophilised
EIA Buffer	A07010	2	Liquid
Concentrated Wash Buffer (10x)	A17012	1	Liquid
Substrate Solution (TMB)	A09010	1	Liquid
Stop Solution	A22000	1	Liquid
Technical Booklet	A11181	1	-
Well cover Sheet	-	1	-

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 41 samples in duplicate.

Precaution for use

Users are recommended to carefully read all instructions for use before starting work.

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- Do not eat, drink or smoke in area in which kit reagents are handled
- Avoid splashing

This kit contains components of human origin. These materials were found non-reactive for HbsAg and for HIV antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents. Wear gloves and laboratory coats are recommended when handling immunodiagnostics materials and samples of human origin.

Stop solution and Substrate solution are potential harmful solution. To avoid any contact, wear eye, hand, face and clothing protection when handling these reagents.

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Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), that is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

Background

Adipocyte fatty acid binding protein AFABP is a 15 kDa member of the intracellular fatty acid binding protein (FABP) family, which is known for the ability to bind fatty acids and related compounds (bile acids or retinoids) in an internal cavity. AFABP is expressed in a differentiation-dependent fashion in adipocytes and is a critical gene in the regulation of the biological function of these cells. In mice, targeted mutations in FABP4 (gen also called: aP2 and its protein also called: P2 adipocyte protein, 3T3-L1 lipid binding protein) provide significant protection from hyperinsulinemia and insulin resistance in the context of both dietary and genetic obesity. Adipocytes obtained from AFABP-deficient mice also have reduced efficiency of lipolysis in vitro and in vivo, and these mice exhibited moderately improved systemic dyslipidemia. Recent studies also demonstrated AFABP expression in macrophages upon differentiation and activation. In these cells, AFABP modulates inflammatory responses and cholesterol ester accumulation, and total or macrophage-specific AFABP deficiency confers dramatic protection against atherosclerosis in the apoE-/- mice. These results indicate a central role for AFABP in the

development of major components of the metabolic syndrome through its distinct actions in adipocytes and macrophages.
Besides being active within the cell, AFAB appears to be a secreted protein. The extracellular role of secreted AFABP remains to be determined.

Principle of the assay

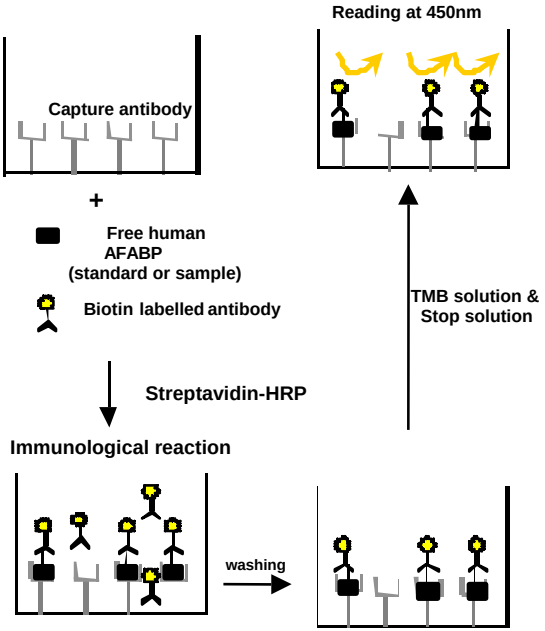
This Enzyme Immunometric Assay (EIA) is based on a double-antibody sandwich technique. The wells of the plate supplied with the kit are coated with a goat polyclonal antibody specific of human AFABP. This antibody will bind any human AFABP introduced in the wells (sample or standard).
After one-hour incubation and a washing, biotin-labelled polyclonal anti-human AFABP antibody is added and incubated with captured AFABP. This allows the two antibodies to form a sandwich by binding on different parts of the human AFABP molecule.

After a thorough wash, streptavidin-horseradish peroxidase tracer is added.

The sandwich is immobilized on the plate so the excess reagents may be washed away. The concentration of the human AFABP is then determined by measuring the enzymatic activity of the HRP using the hydrogen peroxide/TMB solution. The reaction is stopped by addition of sulfuric acid solution. The HRP tracer acts on TMB Reagent to form a yellow compound.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of the human AFABP present in the well during the immunological incubation.

The principle of the assay is summarised below:



Assay characteristics

This kit measures AFABP in serum and plasma (EDTA, citrate, heparin) samples.

- ***Limit of detection (LOD):*** 0.08 ng/mL (defined as the concentration of human AFABP giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + 3*SD_{blank}). The ELISA Buffer was pipetted into blank wells.

- ***Cross-reactivity***

Species	Cross-reactivity
Bovine	no
Cat	no
Dog	yes
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	yes
Pig	no
Rabbit	no
Rat	no
Sheep	no

> *Precision*

- Intra-assay (n=8)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	9.08	0.21	2.3
2	22.75	0.62	2.7

- Inter-assay (n=6)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	31.88	1.74	5.5
2	53.13	1.24	2.3

> *Recovery test (serum samples)*

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	18.63	-	-
	31.87	28.63	111.3
	45.31	43.63	103.9
	69.43	68.63	101.2
2	23.44	-	-
	35.95	33.44	107.5
	52.55	48.44	108.5
	69.84	73.44	95.1

> *Dilution test (serum samples)*

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	-	59.50	-	-
	1:2	29.90	29.75	100.5
	1:4	14.41	14.87	96.8
	1:8	7.57	7.44	101.7
2	-	52.62	-	-
	1:2	26.37	26.31	100.2
	1:4	13.01	13.15	98.9
	1:8	6.77	6.58	102.9

> *Serum / Plasma Samples*

Citrate, EDTA and heparin plasmas were compared to respective serum samples obtained from 10 individuals.

Sample	Mean (ng/mL)	Mean Plasma /Serum (%)	Coefficient of determination R^2
Serum	13.07	-	-
Citrate Plasma	10.33	79.1	0.84
EDTA Plasma	11.38	87.1	0.91
Heparin Plasma	11.58	88.6	0.91

Materials and equipment required

In addition to standard laboratory equipment, the following material is required:

For the assay:

- Precision micropipettes (10 to 1000 µL)
- Spectrophotometer plate reader (450 nm filter) preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Microplate washer (or washbottles)
- Vortex mixer
- Orbital microplate shaker
- Multichannel pipette and disposable tips 100µL
- Deionized (distilled) water
- Polypropylene tubes
- Glassware (graduated cylinder and bottle) for ELISA buffer and Wash buffer

Sample preparation

This assay may be used to measure Human AFABP in serum and plasma samples.

It is the responsibility of the user to check the compatibility of the assay with the study matrix.

General precautions

- All samples must be free from organic solvents prior to assay.
- Samples should be assayed immediately after collection or should be stored at -20°C or at -80°C prior the use with the assay.
- Avoid repeated freeze/thaw cycles, which may cause erroneous results.
- Avoid using hemolyzed or lipemic samples.

Sample preparation

Dilute samples 1/10 in EIA buffer (i.e. 30 µL sample + 270 µL EIA buffer, for duplicates). Mix well; vortex is recommended.
Do not store the diluted samples (1/10).

Reagent preparation

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 41 samples in duplicate.

All reagents need to be brought to room temperature (around +20°C) prior to the assay. Assay reagents are supplied ready to use, except the Standard, Quality Controls, Wash buffer, and Biotin Labelled Antibody.

AFABP (human) Standard

Reconstitute the AFABP (human) Standard vial #A06181 with X µL of EIA Buffer just prior the assay. The volume X is indicated on the vial of the standard. Let it dissolve at least 15 minutes with occasional gentle shaking. Do not make it foam.

The concentration of the human AFABP in the stock solution (S1) is 25 ng/mL. Then prepare the standards as follows:

Standard	Volume of Standard	Volume of EIA Buffer	Standard concentration
S1	-	-	25 ng/mL
S2	200 µL of S1	300 µL	10 ng/mL
S3	250 µL of S2	250 µL	5 ng/mL
S4	250 µL of S3	250 µL	2.5 ng/mL
S5	200 µL of S4	300 µL	1 ng/mL
S6	250 µL of S5	250 µL	0.05 ng/mL

Stability at -20°C: 3 months. Do not store the diluted Standard solutions.

Quality Controls

Reconstitute the vials with X µL (see on the label of QC vial) of distilled water. Mix thoroughly by gentle inversion. Let it dissolve for at least 15 minutes with occasional gentle shaking. Do not make it foam.

Dilute reconstituted Quality Controls 10x with EIA Buffer prior to use (i.e. 30 µL QC + 270 µL EIA buffer).

The reconstituted Quality Controls must be used immediately or stored frozen at -20°C.

Do not store the diluted Quality Controls.
Stability at -20°C: 3 months.

▷ AFABP (human) concentrated Biotin-labelled Antibody (100X)

Prepare the working AFABP (human) Biotin-labelled Antibody solution by diluting 1/100 the AFABP (human) concentrated Biotin-labelled Antibody (100x) with the Biotin-labelled Antibody Dilution Buffer: 10µL of AFABP (human) concentrated Biotin-labelled Antibody (100x) + 990 µL of Biotin-labelled Antibody Dilution Buffer for 1 strip (8 wells).

Do not store the diluted Biotin-labelled Antibody solution.
Stability at 4°C: 3 months.

▷ Concentrated Wash Buffer (10x)

Dilute the Concentrated Wash Buffer (10x) in distilled water to prepare a 1x working solution: 100 mL of Concentrated Wash Buffer + 900 mL of distilled water, for all 96 wells.

Stability at +4°C: 1 month for the diluted Wash solution. Opened Concentrated Wash Buffer (10x) is stable for 3 months.

Substrate solution (TMB)

Substrate Solution (TMB) should remain colourless until added to the plate. Keep substrate solution protected from the light.

Assay procedure

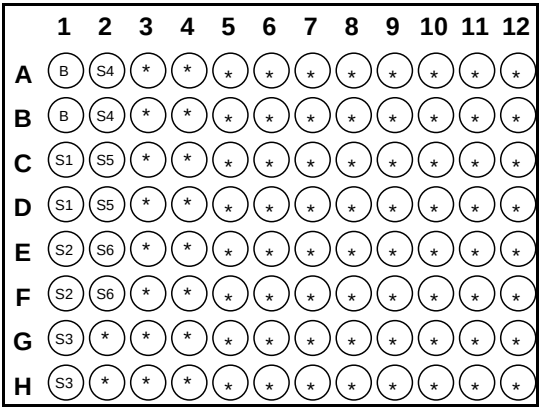
It is recommended to perform the assays in duplicate following the instructions hereafter.

Plate preparation

Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet.
Stability at +4°C: 3 months.

Plate set-up

A plate set-up is suggested hereafter. The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.



B : Blank

S1-S6 : Standards 1-6
* : Samples or Quality Controls

Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.

EIA Buffer

Dispense 100 µL to Blank (B) wells.

AFABP (human) Standard

Dispense 100 µL of each of the Standards (S6 to S1) in duplicates to the appropriate wells.

Start with the lowest concentration standard (S6) and equilibrate the tip in the next higher standard before pipetting.

> *Quality Controls and Samples*

Dispense 100 µL of diluted Quality Controls and samples in duplicate to appropriate wells. Highly concentrated samples may be diluted in ELISA Buffer.

▷ Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

▷ Washing the plate

Rinse each well 5 times with Wash Buffer (350 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents

> ***AFABP (human) Biotin-labelled antibody***

Dispense 100 µL to each well, except Blank (B) wells.

Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Washing the plate

Rinse each well 5 times with Wash Buffer (350 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents

> ***Streptavidin-HRP Tracer***

Dispense 100 µL to each well.

Incubating the plate

Cover the plate with the cover sheet and incubate 30 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Developing and reading the plate

- Empty the plate by turning it over. Rinse each well 5

times with 350 µL of Wash Buffer. At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.

- Add 100 µL of Substrate Solution (TMB) to each well.

- Incubate the plate in the dark at room temperature during 10 minutes. Avoid exposure to direct sunlight. It is recommended to cover the plate with aluminium foil. Do not shake the plate during incubation.

- Add 100 µL of Stop Solution to each well.

- Within 5 minutes following the addition of Stop Solution, read the absorbance of each well at 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 – 650 nm) from the readings at 450 nm.

Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the lowest standard (the highest absorbance of the calibration curve), perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine AFABP concentration of off-scale samples. ***The readings at 405 nm should not replace the on-scale readings at 450 nm.***

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)			
	Blank	Standard	Sample or QC
EIA Buffer	100	-	-
Standard	-	100	-
Sample or QC	-	-	100
Cover plate, incubate **60** minutes at room temperature under orbital shaking at 300 rpm			
Wash strips 5 times with 350 µL/well Discard liquid from the wells & dry on absorbent paper			
AFABP (human) Biotin-labelled Antibody	-	100	
Cover plate, incubate **60** minutes at room temperature under orbital shaking at 300 rpm			
Wash strips 5 times with 350 µL/well Discard liquid from the wells & dry on absorbent paper			
Streptavidin-HRP Tracer	-	100	
Cover plate, incubate **30** minutes at room temperature under orbital shaking at 300 rpm			
Wash strips 5 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper			
Substrate Solution (TMB)	100		
Incubate the plate in the dark without agitation during 10 minutes			
Stop Solution	100		
Read the plate at 450 nm			

Data analysis

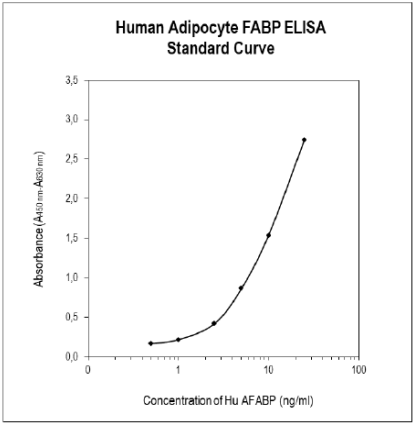
Make sure that your plate reader has subtracted the absorbance readings of the blank well (absorbance of TMB Solution) from the absorbance readings of the rest of the plate. If not, do it at this step.

- Calculate the average absorbance for each Blank, standard, QC and sample.
- For each standard, plot the absorbance on y axis versus the concentration on x axis. Draw a best-fit line through the points.
- To determine the concentration of your samples, find the absorbance value of each sample on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample.
- Most plate readers are supplied with a curve-fitting software capable of graphing these data (4-parameter logistic fit 4PL). If you have this type of software, we recommend using it. Refer to it for further information.

Typical results

The following data are for demonstration purposes only. Your data may be different and still be correct.
The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against log of the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of AFABP ng/ml in samples.

The measured concentration of samples and Quality Controls calculated from the standard curve must be multiplied by their respective dilution factor, because samples and Quality Controls have been diluted prior to the assay. e.g. 1.57 ng/ml (from standard curve) x 10 (dilution factor) = 15.7 ng/ml.



Typical Standard Curve for Human AFABP ELISA

Troubleshooting

Absorbance values are too low:

- one reagent has not been dispensed,
- incorrect preparation or storage of a reagent,
- assay performed before reagents reached room temperature,
- improper wavelength when reading absorbance

High signal and background in all wells:

- inefficient washing,
- overdeveloping (incubation time should be reduced before addition of Stop Solution),
- high ambient temperature.

High dispersion of duplicates:

- poor pipetting technique
- irregular plate washing.

These are a few examples of troubleshooting that may occur. If you need further explanation, Bertin Bioreagent will be happy to assist you. Feel free to contact our Technical Support staff by phone +33 (0)139 306 036, fax +33 (0)139 306 299 or E-mail tech@bertin-bioreagent.com, and be sure to indicate the batch number of the kit (see outside the box).

Bertin Bioreagent proposes ELISA Training kit #B05005 and EIA workshop upon request. For further information, please contact our Technical Support.

Bibliography

Makowski L, Brittingham KC, Reynolds JM, Suttles J and Hotamisligil GS: The Fatty Acid-binding Protein, aP2, Coordinates Macrophage Cholesterol Trafficking and Inflammatory Activity. *J Biol Chem. 2005 Apr 1;280(13):12888-95.*

Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, Cao Q, AtsumiG, Malone H, Krishnan B, Minokoshi Y, Kahn BB, Parker RA and Hotamisligil GS: Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metabolism, Volume 1, Issue 2, February 2005, Pages 107-119.*

Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, Hotamisligil GS: Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice. *Circulation. 2004 Sep 14;110(11):1492-8.*

Lehmann F, Haile S, Axen E, Medina C, Uppenberg J, Svensson S, Lundback T, Rondahl L, Barf T: Discovery of inhibitors of human adipocyte fatty acid-binding protein, a potential type 2 diabetes target. *Bioorg Med Chem Lett. 2004 Sep 6;14(17):4445-8.*

Damcott CM, Moffett SP, Feingold E, Barmada MM, Marshall JA, Hamman RF, Ferrell RE: Genetic variation in fatty acid-binding protein-4 and peroxisome

proliferator-activated receptor gamma interactively influence insulin sensitivity and body composition in males. *Metabolism. 2004 Mar;53(3):303-9.*

Jenkins-Kruchten AE, Bennaars-Eiden A, Ross JR, Shen WJ, Kraemer FB, Bernlohr DA: Fatty acid-binding protein-hormone-sensitive lipase interaction. Fatty acid dependence on binding. *J Biol Chem. 2003 Nov 28;278(48):47636-43.*

Hertzel AV, Bennaars-Eiden A, Bernlohr DA: Increased lipolysis in transgenic animals overexpressing the epithelial fatty acid binding protein in adipose cells. *J Lipid Res. 2002 Dec;43(12):2105-11*.

Fu Y, Luo N, Lopes-Virella MF, Garvey WT: The adipocyte lipid binding protein (ALBP/aP2) gene facilitates foam cell formation in human THP-1 macrophages. *Atherosclerosis. 2002 Dec;165(2):259-69.*

Storch J, Veerkamp JH, Hsu KT: Similar mechanisms of fatty acid transfer from human anal rodent fatty acid-binding proteins to membranes: liver, intestine, heart muscle, and adipose tissue FABPs. *Mol Cell Biochem. 2002 Oct;239(1-2):25-33.*

Fisher RM, Hoffstedt J, Hotamisligil GS, Thorne A, Ryden M: Effects of obesity and weight loss on the expression of proteins involved in fatty acid metabolism in human adipose tissue. *Int J Obes Relat Metab Disord. 2002 Oct;26(10):1379-85.*

Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, Hotamisligil GS: Adipocyte fatty acid-binding protein, aP2, alters late atherosclerotic lesion formation in severe hypercholesterolemia. *Arterioscler Thromb Vasc Biol. 2002 Oct 1;22(10):1686-91.*

Fisher RM, Eriksson P, Hoffstedt J, Hotamisligil GS, Thorne A, Ryden M, Hamsten A, Arner P: Fatty acid binding protein expression in different adipose tissue depots from lean and obese individuals. *Diabetologia. 2001 Oct;44(10):1268-73.*

Scheja L, Makowski L, Uysal KT, Wiesbrock SM, Shimshek DR, Meyers DS, Morgan M, Parker RA, Hotamisligil GS: Altered insulin secretion associated with reduced lipolytic efficiency in aP2-/- mice. *Diabetes. 1999 Oct;48(10):1987-94.*

Coe NR, Simpson MA, Bernlohr DA: Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J Lipid Res. 1999 May;40(5):967-72.*

Baxa CA, Sha RS, Buelt MK, Smith AJ, MatareseV, Chinander LL, Boundy KL and Bernlohr DA: Human adipocyte lipid-binding protein: purification of the protein and cloning of its complementary DNA. *Biochemistry. 1989; 28 (22), 8683-8690.*

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We strive to address a broad range of research interest: inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, pain, prion diseases.

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