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HUMAN AFABP

ENZYME IMMUNOASSAY KIT

catalogue # A05181

96 wells

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For research laboratory use only.
Not for diagnostic use.

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HUMAN AFABP EIA KIT

96 wells
Storage: 2-8℃
Expiry date: stated on the package

This kit contains:

☞A covered 96 well plate, pre-coated with a polyclonal anti-AFABP antibody, ready to use
☞One vial of concentrated biotin labelled anti-AFABP antibody, 100x
☞One vial of biotin labelled antibody buffer
☞One vial of Streptavidin tracer, ready to use
☞One vial of human AFABP standard, lyophilised
☞One vial of Substrate (TMB) solution, ready to use
☞One vial of Stop solution (0.2 M H_2SO_4), ready to use
☞Two vials of EIA Buffer, ready to use
☞Two vials of Quality Controls: low and high, lyophilised
☞One vial of concentrated Wash buffer (10x), liquid
☞One instruction booklet
☞One template sheet
☞One well cover sheet

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.

PRECAUTIONS FOR USE

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

Each time a new pipet tip is used, aspirate a sample of reagent and dispense into the same vessel. Repeat this operation two or three times before distribution.

For research laboratory use only.
Not for diagnostic use.
Do not pipet liquids by mouth.
Do not use kit components beyond the expiration date.
Do not mix different lot numbers.
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.

PRINCIPLE OF THE ASSAY

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.

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).

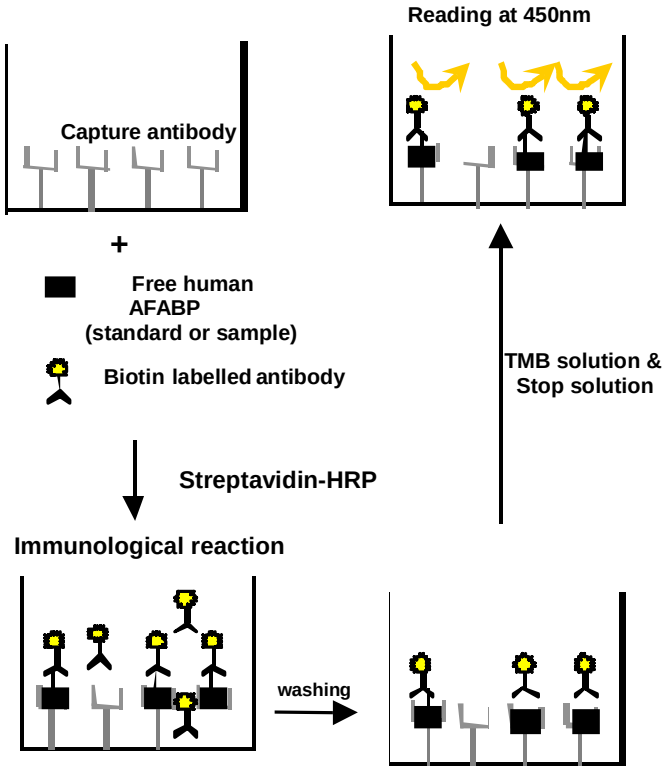
After one-hour incubation and a washing, biotin-labelled polyclonal anti-human AFABP antibody is added and incubated with captured AFABP. This allows the two antibodies to form a sandwich by binding on different parts of the human AFABP molecule.

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

The sandwich is immobilised 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:



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 +/- 10 nm filter)

☞Microtitration washer (or washbottles)

☞Microplate shaker

☞Multichannel pipette 100 µL and disposable tips

☞Distilled or deionised water

☞Polypropylene tubes

SAMPLE PREPARATION

This assay may be used to measure human AFABP in human samples such as serum, plasma (EDTA, citrate, heparin collected blood samples), tissue extract and tissue culture supernatant.

GENERAL PRECAUTIONS

☞All samples must be free of organic solvents prior to assay.

☞Samples should be assayed immediately after collection or should be stored at -20°C, or -70°C for long term storage.

SAMPLE PREPARATION

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

REAGENT PREPARATION

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready to use, except the Standard, Quality Control, Wash buffer.

☞Quality Controls

Reconstitute the vials with xxx µL (see on the label of QC vial) of distilled or deionised water. Mix thoroughly by gentle inversion. Let stand for 25 to 30 minutes.
Dilute reconstituted QC 1/10 in EIA buffer prior to use (i.e. 30 µL QC + 270 µL EIA buffer).
The reconstituted and undiluted QC could be frozen at -20°C until next use.
Do not store the diluted (1/10) QC.

☞Human AFABP Standards

Reconstitute AFABP standard with xxx µL of EIA Buffer (see on the label of standard vial). The concentration of the human AFABP in the stock solution (S1) is 25 ng/mL. Then prepare standards as follows:

Volume of Standards	Added volume of EIA buffer	Concentration of reconstituted standards
500 µL of S1	750 µL	S2 (10 ng/mL)
500 µL of S2	500 µL	S3 (5 ng/mL)
500 µL of S3	500 µL	S4 (2.5 ng/mL)
500 µL of S4	750 µL	S5 (1 ng/mL)
500 µL of S5	500 µL	S6 (0.5 ng/mL)

The reconstituted standards are ready to use: do not dilute them. They could be frozen at -20°C until next use.

☞Biotin labelled Antibody solution

Prepare the working Biotin labelled Antibody solution by diluting 1/100 the concentrated biotin labelled antibody (100x) with the biotin labelled antibody buffer : 110µL concentrated antibody in 10890µL antibody buffer

Concentrated biotin labelled antibody solution is stable for 3 months at +4°C.

Do not store the diluted biotin labelled antibody solution.

☞Wash buffer

Reconstitute one vial of concentrated Wash buffer (100 mL, 10x) to 1000 mL with distilled or deionised water.

☞Hydrogen peroxide/TMB solution

Substrate solution 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 and to follow the instructions hereafter.

DISTRIBUTION OF REAGENTS AND SAMPLES

A plate set-up is suggested on this page. The content of each well may be recorded on the sheet provided with the kit.

PIPETTING THE REAGENTS

All samples and reagents must reach room temperature prior performing the assay. Use different tips to pipet the buffer, standard, sample, tracer antiserum and other reagents.

- Human AFABP standard:

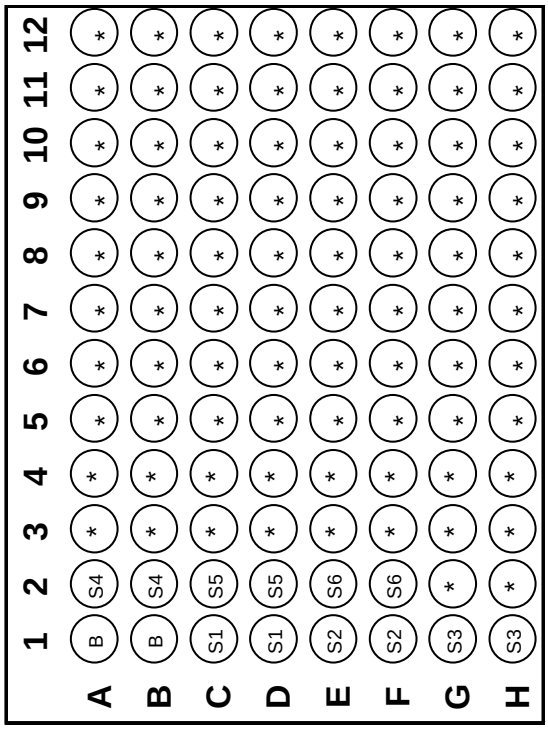
  Dispense 100 µL of each reconstituted standard (S1 to S6 : 0.5 to 25 ng/mL) in duplicate to appropriate wells. Start with the lowest concentration standard and equilibrate the tip in the next higher standard before pipetting.

- Quality Control and samples:

  Dispense 100 µL of diluted Quality Control and samples in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA buffer.

- EIA buffer:

  Dispense in duplicate 100 µL of EIA buffer in Blank (B) wells.



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

INCUBATING THE PLATE

- Cover the plate with the cover sheet and incubate the plate for 1 hour at R.T., shaking at 300 rpm on an orbital microplate shaker.
- Rinse the wells 5 times with the Wash buffer (350 µL per well). After the final wash, invert the plate and tap it strongly against paper towel.
- Biotin-labelled anti-AFABP antibody solution: Dispense 100 µL to each well.
- Cover the plate with the cover sheet and incubate the plate for 1 hour at R.T., shaking at 300 rpm on an orbital microplate shaker.
- Rinse the wells 5 times with the wash buffer (350 µL per well). After the final wash, invert the plate and tap it strongly against paper towel.
- Streptavidin-HRP conjugate: Dispense 100 µL to each well.
- Cover the plate with the cover sheet and incubate the plate for 30 minutes at R.T., shaking at 300 rpm on an orbital microplate shaker.
- Rinse the wells 5 times with the wash buffer (350 µL per well). After the final wash, invert the plate and tap it strongly against paper towel.

DEVELOPING AND READING THE PLATE

- Dispense 100 µL of Substrate solution to the 96 wells. Incubate in the dark during 10 minutes at room temperature (20-30 ℃). Avoid exposure to direct sunlight. It is recommended to cover the plate with aluminium foil.
- Stop the colour development by adding 100 µL of Stop solution.
- Read the absorbance at 450 nm within 5 minutes following stop solution addition.

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.***

Enzyme Immunoassay Protocol (Volumes are in µL)			
	Blank	Standard	Sample
EIA Buffer	100		
Standard	-	100	-
Sample	-	-	100
Incubate the plate at room temperature for 1 hour			
Wash the plate 5 times			
Biotin Labelled AFABP antibody	-	100	100
Incubate the plate at room temperature for 1 hour			
Wash the plate 5 times			
Streptavidin-HRP	100	100	100
Incubate the plate at room temperature for 30 minutes			
Wash the plate 5 times			
TMB solution	100	100	100
Incubate the plate in the dark at room temperature during 10 minutes			
Stop solution	100	100	100
Read the plate at 450 nm			

DATA ANALYSIS

Make sure that your plate reader has substracted 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 now.

- Using a logit-log graph paper, plot the absorbance for each standard (y axis) versus concentration (x axis) of standards. Draw a best-fit line through the points.

- To determine the concentration of your samples, find the absorbance value 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 curve-fitting software capable of graphing this type of data (logit/log or 4-parameter). If you have this type of software, we recommend using it. Refer to it for further information.

TYPICAL DATA

EXAMPLE DATA

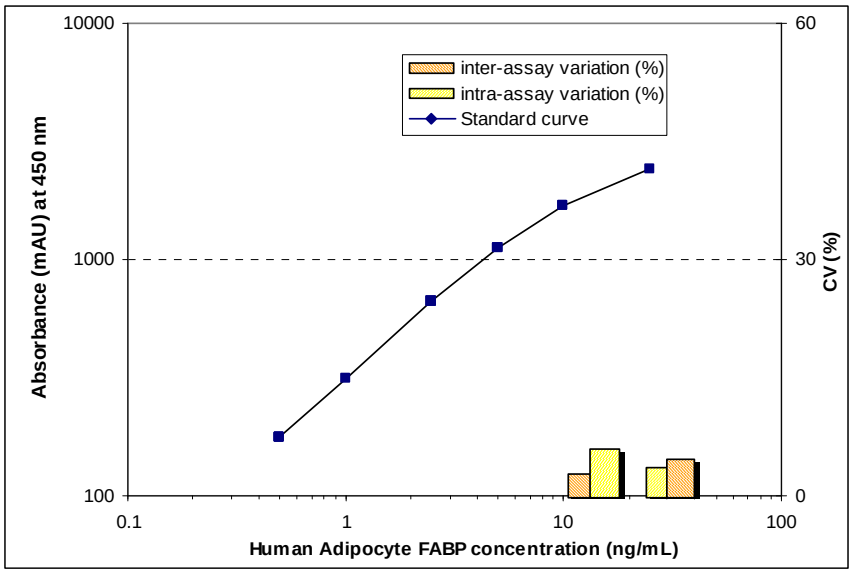
The following data are for demonstration purposes only. Your data may be different but still be correct. These data were obtained using all reagents as supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Human AFABP	mAU
Blank	12
Standard 25ng/mL	2 434
Standard 10 ng/mL	1 682
Standard 5 ng/mL	1 121
Standard 2.5 ng/mL	691
Standard 1 ng/mL	313
Standard 0.5 ng/mL	178
QC High	859
QC Low	253

ACCEPTABLE RANGE

☞QC samples: see label on the vials.

HUMAN AFABP STANDARD CURVE



ASSAY VALIDATION AND CHARACTERISTICS

The Enzyme Immunometric assay of human AFABP has been validated for its use in human samples such as serum, plasma and tissue extract and tissue culture supernatant.

☞Cross-reactivity:

- Human Leptin	<0.1%
- Human Leptin Receptor	<0.1%
- Human Resistin	<0.1%
- Monkey serum equivalent to:	19 ng/mL
- Rabbit AFABP	<0.1%
- Goat AFABP	<0.1%
- Pig AFABP	<0.1%
- Horse AFABP	<0.1%
- Rat AFABP	<0.1%
- Sheep AFABP	<0.1%
- Hamster AFABP	<0.1%
- Bovine AFABP	<0.1%
- LAFABP (liver, FABP1)	<0.2%
- IFABP (intestinal, FABP2)	<0.2%
- HFABP (heart, muscle, FABP3)	<0.2%
- EFABP (epithelial, epidermal, FABP5)	<0.2%
- Gastrotropin (ileal FABP, FABP6)	<0.2%

☞Sensitivity:

The limit of detection (defined as such a concentration of human AFABP-1 giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + 3*SD_{blank}) is better than 0.1 ng/mL of sample. The EIA buffer was pipetted into blank wells, and the microtiter plate is blanked on air.

☞Precision:

- Intra-assay (n=8)

Sample	Mean (ng/mL)	Standard Deviation (ng/mL)	CV (%)
1	13.9	0.92	6.6
2	27.3	1.08	3.9

- Inter-assay (Run-to-Run, n=3)

Sample	Mean (ng/mL)	Standard Deviation (ng/mL)	CV (%)
1	12.5	0.32	2.6
2	31.1	1.58	5.1

☞Recovery test:

Serum samples were spiked with different amounts of human AFABP, diluted 1/10 in EIA buffer and assayed.

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	8.9	-	-
	16.5	18.9	87.3
	24.1	28.9	83.4
	55.55	58.9	94.2
2	6.8	-	-
	15.4	16.8	91.7
	24.2	26.8	90.3
	53.1	36.8	93.5

☞Dilution test:

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	-	36.8	-	-
	2x	19.6	18.4	106.5
	4x	9.9	9.2	107.6
	8x	4.9	4.6	106.5
2	-	28.1	-	-
	2x	14.1	14.1	100.0
	4x	7.8	7.0	111.0
	8x	3.9	3.5	111.0

☞Effect of sample matrix (serum/plasma):

Samples from 10 healthy individuals were taken and treated by different methods (serum, citrate plasma, EDTA plasma and heparin plasma), results shown below:

Volunteer	Serum (ng/mL)	Plasma (ng/mL)		
		EDTA	Citrate	Heparin
1	29,8	29,2	26,7	26,8
2	49,8	55,1	42,6	52,8
3	34,7	36,7	34,7	36,4
4	52,3	47,3	35,6	45,9
5	55,8	48,3	41,4	49,8
6	29,9	27,3	25,8	29,5
7	43,1	39,9	37,8	41,3
8	23,9	20,5	17,0	20,4
9	29,8	26,0	23,1	27,3
10	25,7	24,2	20,4	25,8

☞Serum/ Plasma Samples:

Mean values of human AFABP in serum, heparin plasma, citrate plasma and EDTA plasma:

Sample (n = 10)	Mean AFABP (ng/mL)	Plasma /Serum (%)
Serum	37.48	-
Heparin Plasma	35.60	95.0
Citrate Plasma	30.51	81.4
EDTA Plasma	35.45	94.6

☞Effect of freezing/thawing on the concentration of AFABP in samples:

No significant decline was observed in concentration of AFABP in serum or plasma samples after repeated (3x) freezing/thawing cycles.

Volunteer	Number of f/t cycles	Serum ng/mL	EDTA Plasma (ng/mL)	Heparin Plasma (ng/mL)	Citrate Plasma (ng/mL)
1	1x	52,0	46,2	51,4	43,7
	3x	53,5	47,9	47,2	41,8
	5x	49,8	44,3	46,1	40,9
2	1x	62,2	62,1	61,8	57,1
	3x	64,6	60,5	62,8	53,8
	5x	61,3	57,8	55,8	52,5
3	1x	30,3	32,8	32,9	27,3
	3x	32,9	31,7	36,8	27,6
	5x	34,5	31,7	33,3	27,6

☞Stability of samples at +4℃:

Samples should be stored at -20℃. However, no decline was observed in concentration of AFABP in serum and plasma samples when stored at 4℃ for 2 weeks. To avoid microbial contamination add NaN3 to a final concentration 0.1% to the samples.

ASSAY TROUBLE SHOOTING

☞Absorbance values too low:
- One reagent has not been dispensed
- Incorrect preparation or reagent storage
- Assay performed before reagents reach room temperature

☞High signal and background in all wells:
- Inefficient washing
- Overdeveloping; incubation time should be reduced before adding Stop Solution

☞High dispersion of duplicates:
- Poor pipetting technique or irregular plate washing.

These are a few examples of problems that may occur. If you need further assistance, SPI-BIO will be happy to answer any questions or information about this assay. Please feel free to contact our technical support staff by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (sales@spibio.com), and be sure to indicate the lot number of the kit (see outside of the box).

SPI-BIO offers a training workshop in EIA practice & theory. This workshop is given twice a year. For further information, please contact our Customer Relation Representative (33 (0)1 39 30 62 60).

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