DRG® Adipocyte FABP (human) ELISA (EIA-4908)

RUO

As of 25 Feb. 2010 rm (Vers. 1.0)

1 INTENDED USE

The Adipocyte FABP (human) ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human AFABP.

Features

- **It is intended for research use only.**
- The total assay time is less than 4 hours.
- The kit measures total AFABP in serum, plasma (EDTA, citrate, heparin).
- Assay format is 96 wells.
- Quality Controls are human serum based. No animal sera are used.
- Standard is recombinant protein based.
- Components of the kit are provided ready to use, concentrated or lyophilized.

2 STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).
For stability of opened reagents see Chapter 9.

3 INTRODUCTION

Protein definition:

Protein name:	Adipocyte FABP (AFABP)
Synonyms:	Fatty acid-binding protein, adipocyte (A-FABP) Adipocyte lipid-binding protein (ALBP)
Gene name:	FABP4
Swissprot:	P15090

4 NCBI / PROTEIN: P15090

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 (gene 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, AFABP appears to be a secreted protein (for normal levels and correlations with certain metabolic parameters see chapter 15). The extracellular role of secreted AFABP remains to be determined.

Areas of investigation:

Energy metabolism and body weight regulation

5 TEST PRINCIPLE

In the Adipocyte FABP (human) ELISA, Standards, Quality Controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human AFABP antibody. After 60 minutes incubation and washing, biotin-labelled polyclonal anti-human AFABP antibody is added and incubated for 60 minutes with captured AFABP. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured spectrophotometrically at 450 nm.

The absorbance is proportional to the concentration of AFABP. A standard curve is constructed by plotting absorbance values against concentrations of Standards, and concentrations of unknown samples are determined using this standard curve

6 PRECAUTIONS

For professional use only.

Wear gloves and laboratory coats when handling immunodiagnostic materials.

Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.

This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.

Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.

The materials must not be pipetted by mouth.

7 TECHNICAL HINTS

Reagents with different lot numbers should not be mixed.

Use thoroughly clean glassware.

Use deionized (distilled) water, stored in clean containers.

Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.

Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light.

Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

8 REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody	ready to use	13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	1 vial
Quality Control High	lyophilized	1 vial
Quality Control Low	lyophilized	1 vial
Dilution Buffer	ready to use	2 x 13 ml
Wash Solution Concentrate (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis		1 pc

9 MATERIAL REQUIRED BUT NOT SUPPLIED

Deionized (distilled) water
Test tubes for diluting samples
Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
Precision pipettes to deliver 10-1 000 µL with disposable tips
Multichannel pipette to deliver 100 µL with disposable tips
Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
Vortex mixer
Orbital microplate shaker capable of approximately 300 rpm
Microplate washer (optional). [Manual washing is possible but not preferable.]
Microplate reader with 450 ± 10 nm filter
Software package facilitating data generation and analysis (optional)

10 PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use.

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on their label.

Assay reagents supplied ready to use:

– **Antibody Coated Microtiter Strips**

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

- **Biotin Labelled Antibody**
- **Streptavidin-H RP Conjugate**
- **Dilution Buffer**
- **Substrate Solution**
- **Stop Solution**

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

Assay reagents supplied concentrated or lyophilized:

- **Human AFABP Master Standard**

Refer to Certificate of Analysis for actual volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasionally gently shaking (not to foam). The resulting concentration of the Human AFABP in the stock solution is **25 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Standard Diluent	Concentration
Stock	-	25 ng/ml
500 µL of stock	750 µL	10 ng/ml
500 µL of 10 ng/ml	500 µL	5 ng/ml
500 µL of 5 ng/ml	500 µL	2.5 ng/ml
500 µL of 2.5 ng/ml	750 µL	1 ng/ml
500 µL of 1 ng/ml	500 µL	0.5 ng/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage:

Standard stock solution (25 ng/ml) should be aliquoted and frozen at –20°C for 3 months.
Avoid repeated freeze/thaw cycles.
Do not store the diluted Standard Solutions.

- **Quality Controls High, Low**

Refer to the Certificate of Analysis for actual volume of distilled water for reconstitution and for actual Quality Controls concentrations!!!

Reconstitute each Quality Control (High and Low) with distilled water just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Dilute reconstituted Quality Controls 10x with Dilution Buffer, e.g. 20 µL of Quality Control + 180 µL of Dilution Buffer when assaying samples in singlets, or preferably 30 µL of Quality Control + 270 µL of Dilution Buffer for duplicates.

Stability and storage:

The reconstituted Quality Controls must be used immediately or stored frozen at -20°C for 3 months.
Avoid repeated freeze/thaw cycles.
Do not store the diluted Quality Controls.

– **Wash Solution**

Dilute Wash Solution Concentrate (10x) ten-fold in 900 ml of distilled water to prepare a 1x working solution.

Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2-8°C.
Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

11 PREPARATION OF SAMPLES

The kit measures AFABP in serum, plasma.

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples just prior to the assay 10x with Dilution Buffer,
e.g. 20 µL of sample + 180 µL of Dilution Buffer for singlets, or preferably 30 µL of sample + 270 µL of Dilution Buffer for duplicates.
Mix well (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored at -20°, or preferably at -70°C for long-term storage.
Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

See Chapter 13 for stability of serum and plasma samples if stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of AFABP.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

Ask for protocol at DRG if assaying tissue extract, urine, breast milk, or tissue culture medium samples.

12 ASSAY PROCEDURE

1. Pipet **100 µL** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour,** shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells **5-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µL** of Biotin Labeled Antibody into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour,** shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells **5-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.

7. Add **100 µL** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes,** shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells **5-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µL** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µL** of Stop Solution.
13. Determine the absorbance by reading the plate at 450 nm.
    The absorbance should be read within 5 - 15 minutes following step 12.

Note: If the micro plate reader is not capable of reading absorbance greater than the absorbance of the highest standard, 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.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

13 CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance at 450 nm (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.

Alternatively, the logit log function can be used to linearize the standard curve (i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

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.

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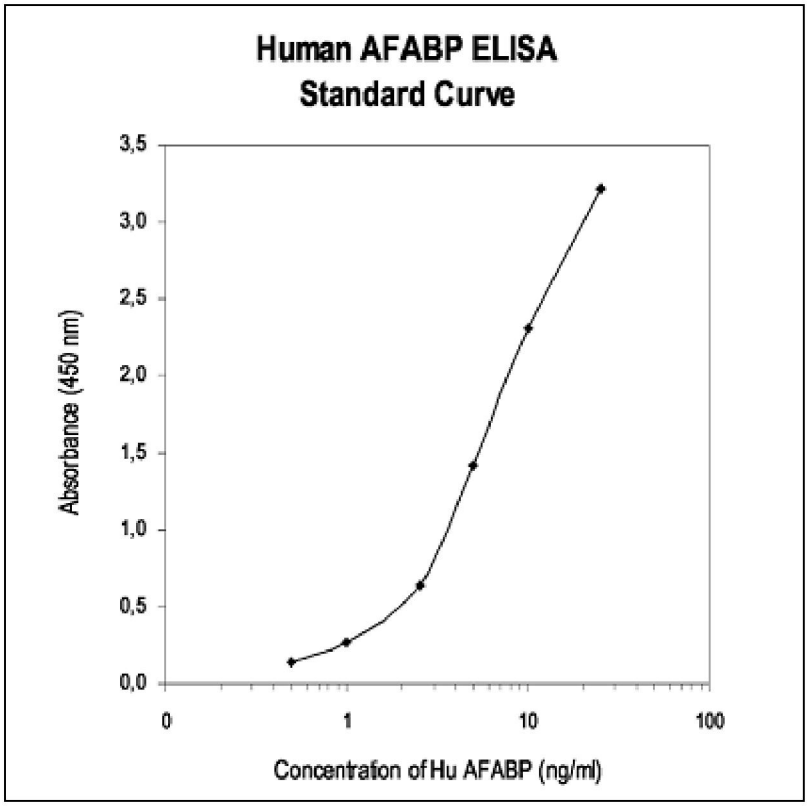

Figure 1: Typical Standard Curve for Adipocyte FABP (human) ELISA.

14 PERFORMANCE CHARACTERISTICS

Typical analytical data of Adipocyte FABP (human) ELISA are presented in this chapter.

14.1 Sensitivity

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is calculated from the real AFABP values in wells and is less then 0.1 ng/ml.

*Dilution Buffer is pipetted into blank wells.

14.2 Limit of assay

Results exceeding AFABP level of 25 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the AFABP concentration.

14.3 Specificity

The antibodies in Adipocyte FABP (human) ELISA kit are highly specific for human AFABP with no detectable cross reactivities to human leptin, leptin receptor, adiponectin, resistin, HFABP, LFABP, IFABP, EFABP and RELM-beta at 50 ng/ml and IL-6, AGRP, ASP (C3adesArg) at 2 ng/ml.

Sera of several mammalian species were measured in the assay. See results below.

Mammalian serum sample	Observed Cross reactivity
Bovine	no
Cat	yes
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	yes
Pig	no
Rabbit	yes
Rat	yes
Sheep	no

14.4 Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	14.92	0.13	8.98
2	38.52	0.20	5.24

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	14.56	0.045	0.31
2	33.73	1.009	2.99

14.5 Spiking Recovery

Serum samples were spiked with different amounts of human AFABP, diluted with Dilution Buffer 10x and assayed.

Sample	Observed	Expected	Recovery O/E
	(ng/ml)	(ng/ml)	(%)
1	17.10	-	-
	30.45	27.10	112.4
	40.36	37.10	108.8
	49.90	47.10	105.9
2	17.13	-	-
	27.64	27.13	101.9
	37.56	37.13	101.2
	48.58	47.13	103.1

14.6 Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

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

14.7 Effect of sample matrix

Citrate, heparin and EDTA plasmas were compared to respective serum samples from the same 10 individuals. Results are shown below:

Volunteer No.	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	37.5	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
Mean (ng/ml)	**37.48**	**35.45**	**30.50**	**35.60**
Mean Plasma/Serum (%)	**-**	**94.6**	**81.4**	**95.0**
Correlation. coeff. R^2	**-**	**0.91**	**0.84**	**0.93**

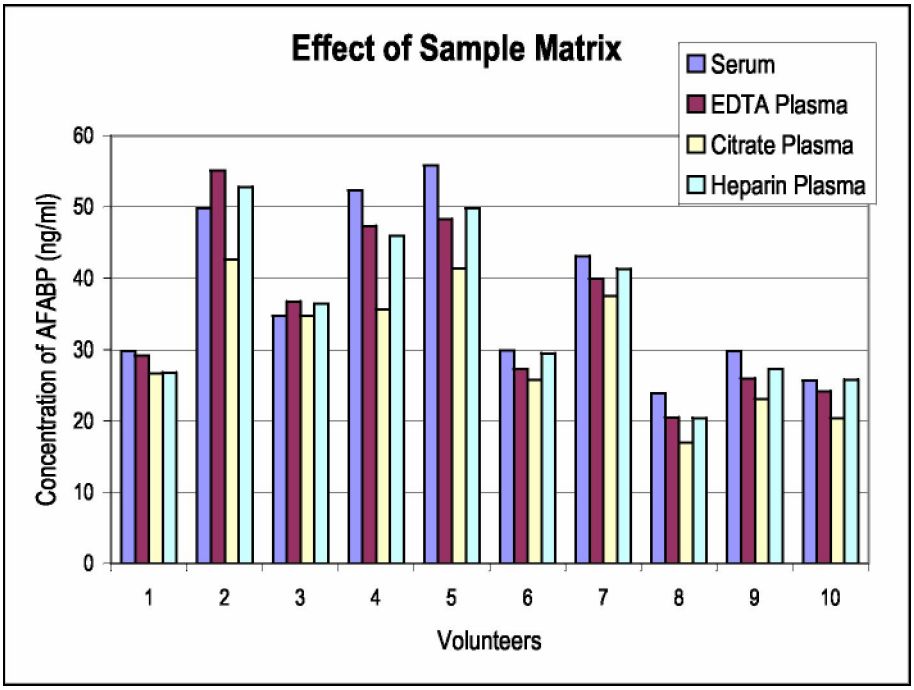

Figure 2: AFABP levels measured using Adipocyte FABP (human) ELISA from 10 individuals using serum, ED TA, citrate and heparin plasma, respectively.

14.8 Stability of samples stored at 2-8C

Samples should be stored at –20°C. However, no decline in concentration of AFABP was observed in serum and plasma samples after 14 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with å-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

14.9 Effect of Freezing/Thawing

No decline was observed in concentration of human AFABP in serum and plasma samples after repeated (3x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
1	1x	52.0	46.2	43.7	51.4
	3x	53.5	47.9	41.8	47.2
	5x	49.8	44.3	40.9	44.3
2	1x	62.2	62.2	61.8	57.1
	3x	64.6	60.6	53.8	62.8
	5x	61.3	57.8	52.5	55.8
3	1x	30.3	32.8	27.3	32.9
	3x	32.9	31.7	27.6	36.8
	5x	34.5	31.7	27.6	33.3

15 DEFINITION OF THE STANDARD

The recombinant protein is used as the standard in this assay. The recombinant AFABP is a 14.7 kDa protein containing 132 amino acid residues. Master Standard used in this kit contains 25 ng of AFABP measured by BCA method (Sigma-Aldrich).

16 PRELIMINARY POPULATION AND CLINICAL DATA

In our preliminary study, we investigated relations between serum AFABP value and some basic population parameters, parameters of lipid metabolism and parameters of insulin sensitivity.

16.1 Normal value and normal range in human serum

The mean value study with sera samples from 66 random selected women, 35-52 years old, has been established with the Adipocyte FABP (human) ELISA in our laboratory (n=66, mean ± SEM): 19.58 ± 1.01 ng/ml.

The normal range with sera samples from 66 random selected women, 35-52 years old, has been established with the Adipocyte FABP (human) ELISA in our laboratory: Normal range (mean ± 2 SD): 19.58 ± 16.32 ng/ml.

However, it is recommended to establish a normal range control serum for each laboratory. The presented reference range should be regarded as guideline only.

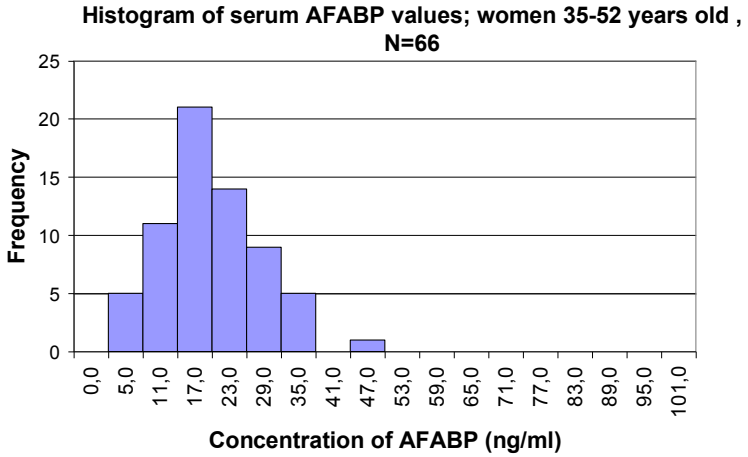

Figure 3: Histogram of serum AFABP values shows Gaussian distribution.

16.2 Influence of gender on AFABP value in human serum

The comparison of serum AFABP values between men (n=1 0) and women (n=26) selected by age (35-52 years old volunteers) did not show any significant difference.

Results are presented as mean values:

	Age (years)	Total cholesterol (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	Triglycerides (mmol/l)	AFABP (mmol/l)
Women	45.5	5.21	1.46	3.35	1.13	21.28
Men	43.7	5.08	1.06	3.70	1.61	21.44

16.3 Clinical relevance of serum AFABP value

We have found correlations between serum AFABP value and triglycerides, HDL and LDL values.

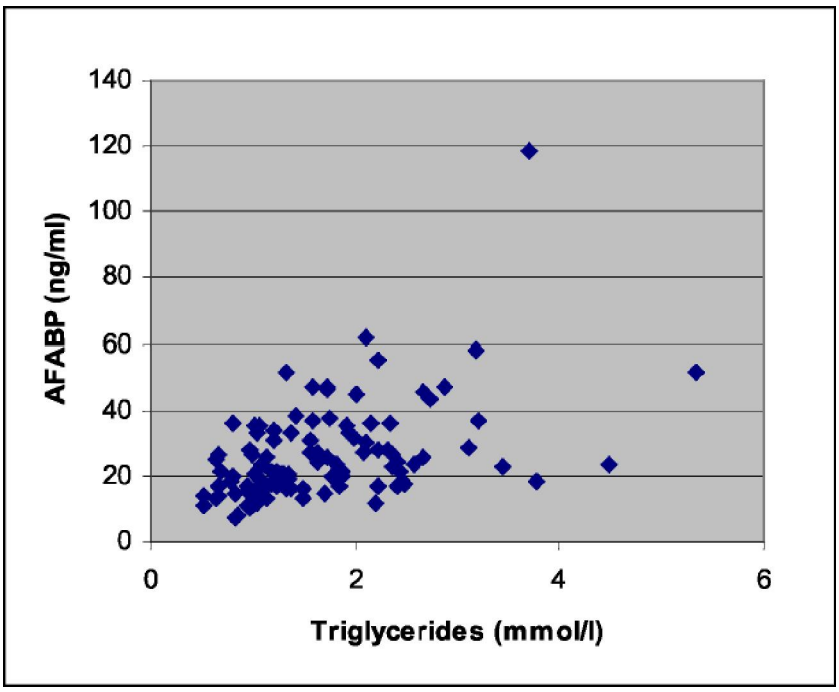

Figure 4: Serum AFABP positively correlates with triglycerides

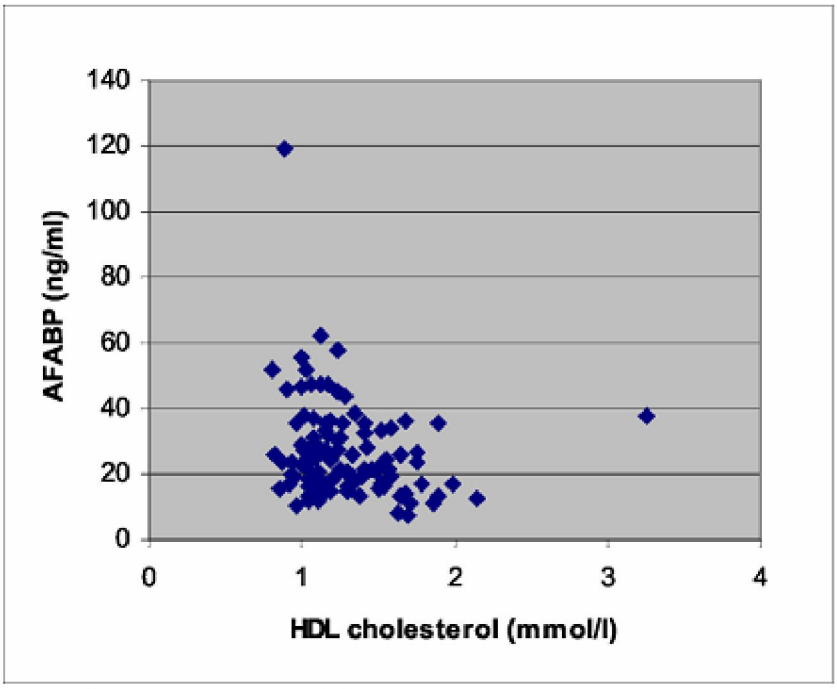

Figure 5: Serum AFABP negatively correlates with HDL

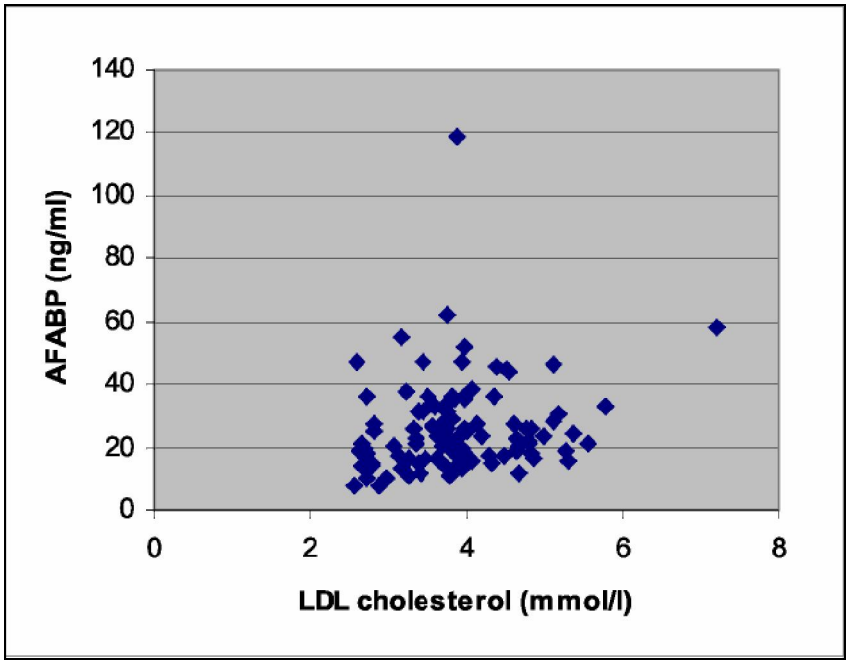

Figure 6: Serum AFABP positively correlates with LDL

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Increased serum AFABP values were found in obese volunteers in a similar study. AFABP values are presented as mean +/- SD:

	Volunteer No. (F/M)	Age (years)	AFABP (ng/ml)
Obese (BMI > 25)	129 (62/67)	53.9 ± 12.7	32.3 ± 14.8
Non-obese (BMI < 25)	100 (46/54)	57.6 ± 12.8	20.0 ± 9.8

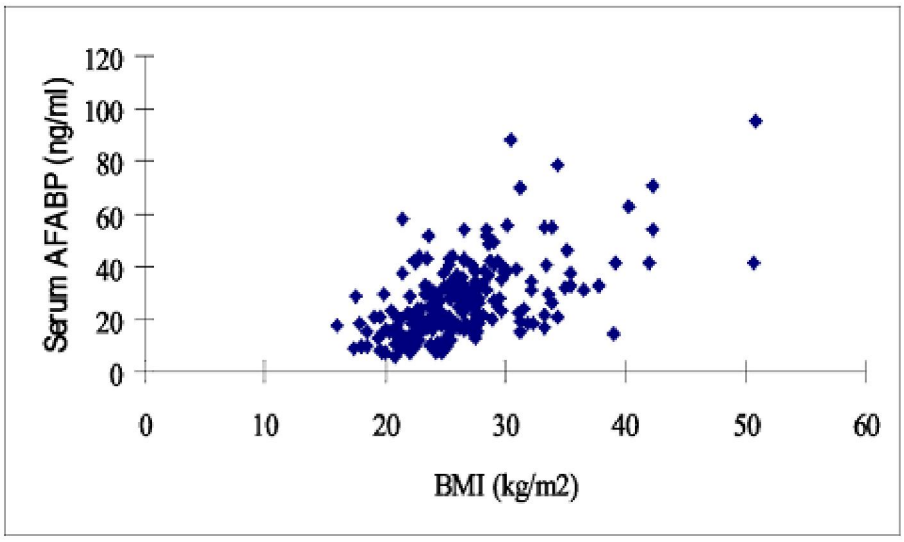

Figure 7: Serum AFABP correlates with BMI

Moreover, serum AFABP is related to insulin sensitivity:

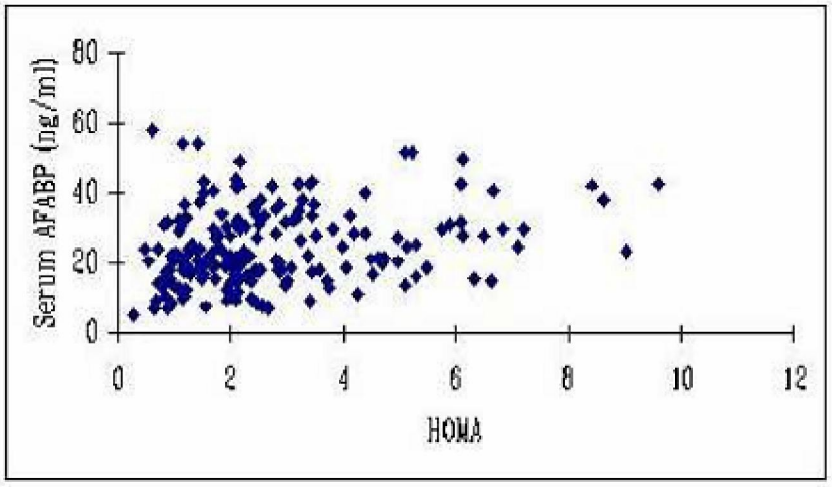

Figure 8: Serum AFABP correlates with HOMA score

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological references ranges for AFABP levels with the assay.

17 METHOD COMPARISON

Since no competitive commercial test is available, Adipocyte FABP (human) ELISA has not been compared to any other immunoassay.

18 TROUBLESHOOTING AND FAQS

– **Weak signal in all wells**

Possible explanations:

Omission of a reagent or a step
Improper preparation or storage of a reagent
Assay performed before reagents were allowed to come to room temperature
Improper wavelength when reading absorbance

– **High signal and background in all wells**

Possible explanations:

Improper or inadequate washing
Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
Incubation temperature over 30°C

– **High coefficient of variation (CV)**

Possible explanation:

Improper or inadequate washing
Improper mixing Standards, Quality Controls or samples

19 REFERENCES

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IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
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