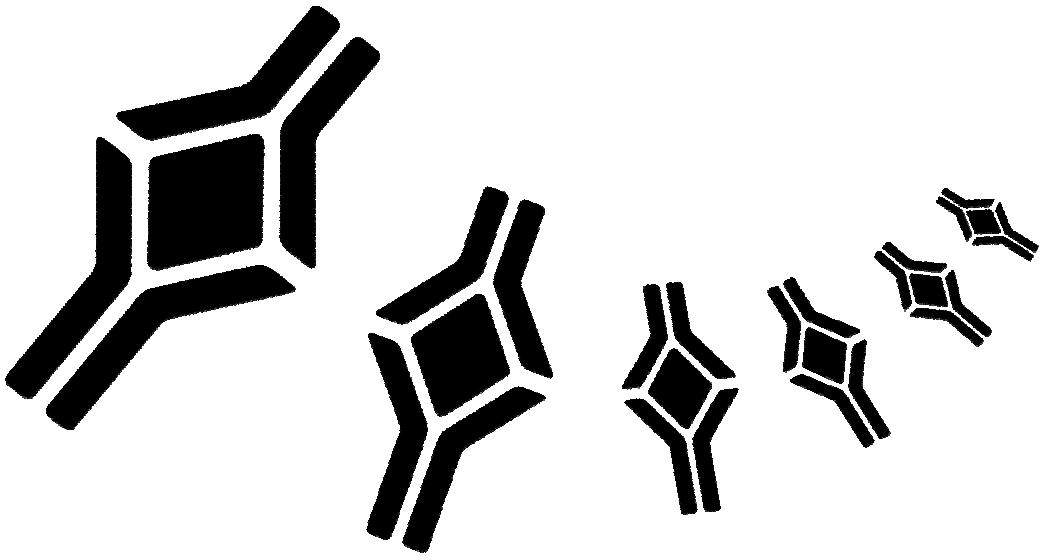
BioVendor
Research
and Diagnostic Products



HUMAN ADIPOCYTE FABP ELISA (HUMAN FABP4 ELISA)

Product Data Sheet

Cat. No.: RD191036200R

For Research Use Only

VERSION 03 150311 19

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RD191036200R Human AFABP 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 and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized
- Patent Application Number: DE 10 2005 034 788.6

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
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 (mouse gene is also called aP2 and its relevant protein P2 adipocyte protein or 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 human 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

4. TEST PRINCIPLE

In the BioVendor Human AFABP 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 . 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.

5. 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
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- 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

6. 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

7. 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 Conc. (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

8. 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 10-1000 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, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. 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-HRP 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 the Certificate of Analysis for current 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 occasional gentle 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	*Dilution Buffer*	*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 current volume of distilled water needed for reconstitution and for current Quality Control concentration!!!
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 Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in 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.

10. PREPARATION OF SAMPLES

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

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 serum or plasma samples 10x with Dilution Buffer just prior to the assay, 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 when 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.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
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 Labelled 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 of each well using a microplate reader set to 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. **The absorbance should be read within 5 minutes following step 12.**

Note: If the microplate 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.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 25**	**QC HIGH**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 10**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 5**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 2.5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 1**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 0.5**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC LOW**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. 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.

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.

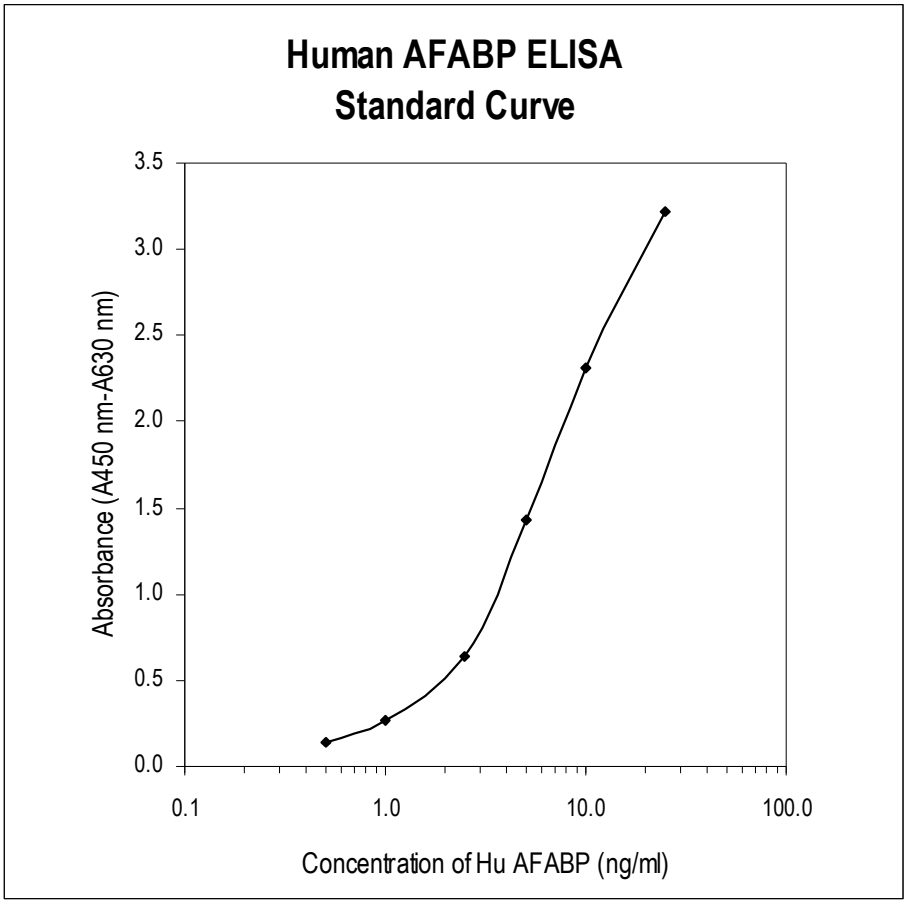


Figure 2: Typical Standard Curve for Human AFABP ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human AFABP ELISA are presented in this chapter.

- **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 0.1 ng/ml.

*Dilution Buffer is pipetted into blank wells.

- **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.

- **Specificity**

The antibodies in Human AFABP ELISA kit are highly specific for human AFABP with no detectable crossreactivities 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.
For details please contact us at info@biovendor.com.

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

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (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)*	*SD (ng/ml)*	*CV (%)*
1	12.5	0.32	2.6
2	31.1	1.58	5.1

- **Spiking Recovery**

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

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
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

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (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**

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**
Coefficient of determination R^2	**-**	**0.91**	**0.84**	**0.93**

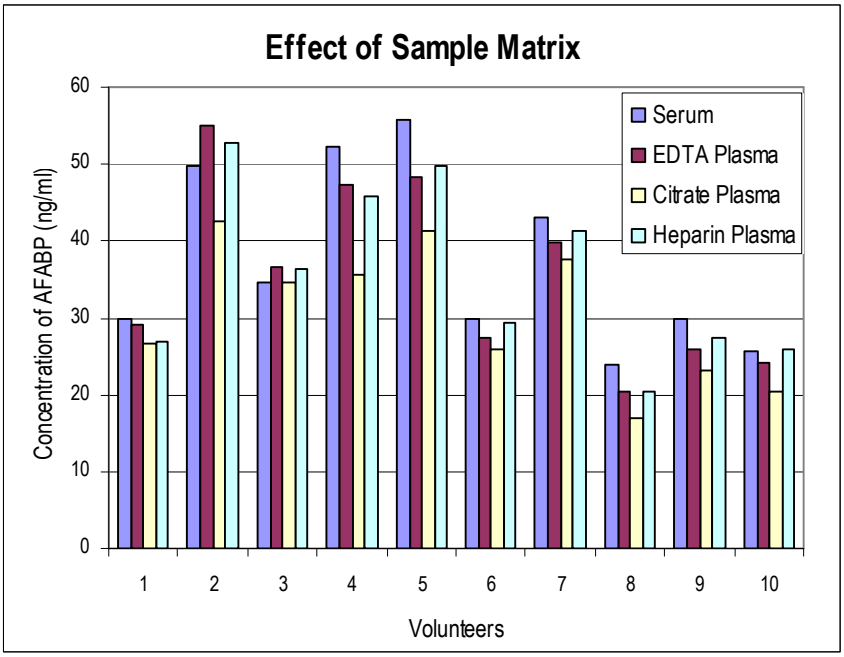


Figure 3: AFABP levels measured using Human AFABP ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **Stability of samples stored at 2-8°C**

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 ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human AFABP in serum and plasma samples after repeated (5x) 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	57.1	62.1
	3x	64.6	64.6	53.8	60.5
	5x	61.3	61.3	52.5	57.8
3	1x	30.3	30.3	27.3	32.8
	3x	32.9	32.9	27.6	36.8
	5x	34.5	34.5	27.6	33.3

14. 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).

15. 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.

- **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 Biovendor Human AFABP 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 Biovendor Human AFABP ELISA in our laboratory:
Normal range (mean ± 2 SD): **19.58 ± 16.32 ng/ml.**

- **Reference range**

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.

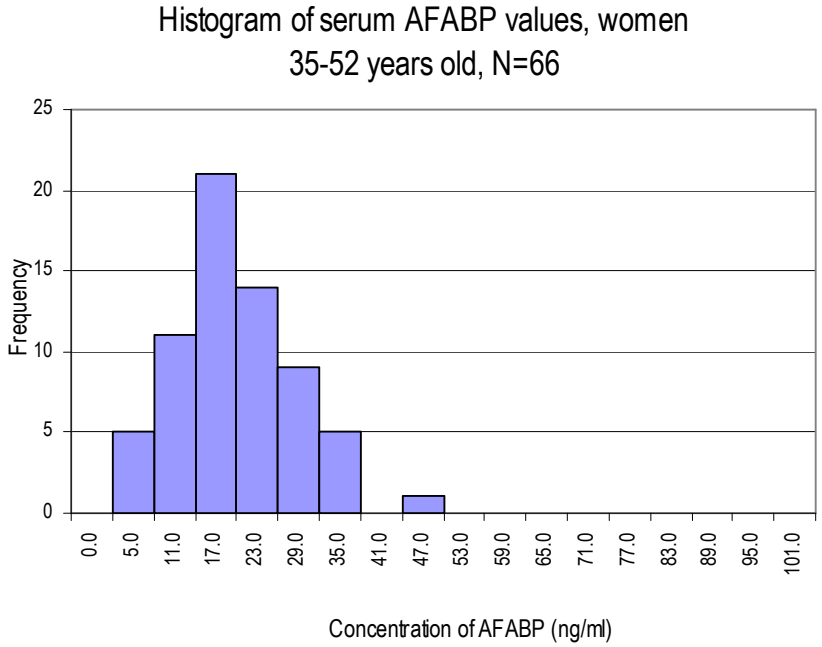


Figure 4: Histogram of serum AFABP values shows Gaussian distribution.

- **Influence of gender on AFABP value in human serum**

The comparison of serum AFABP values between men (n=10) 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

- **Clinical relevance of serum AFABP value**

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

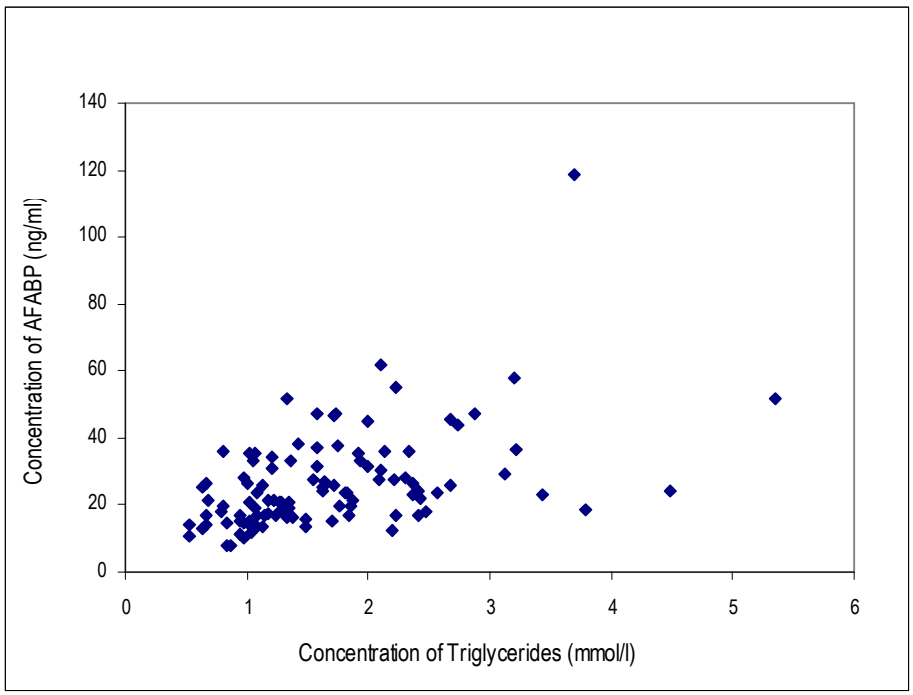


Figure 5: Serum AFABP positively correlates with triglycerides.

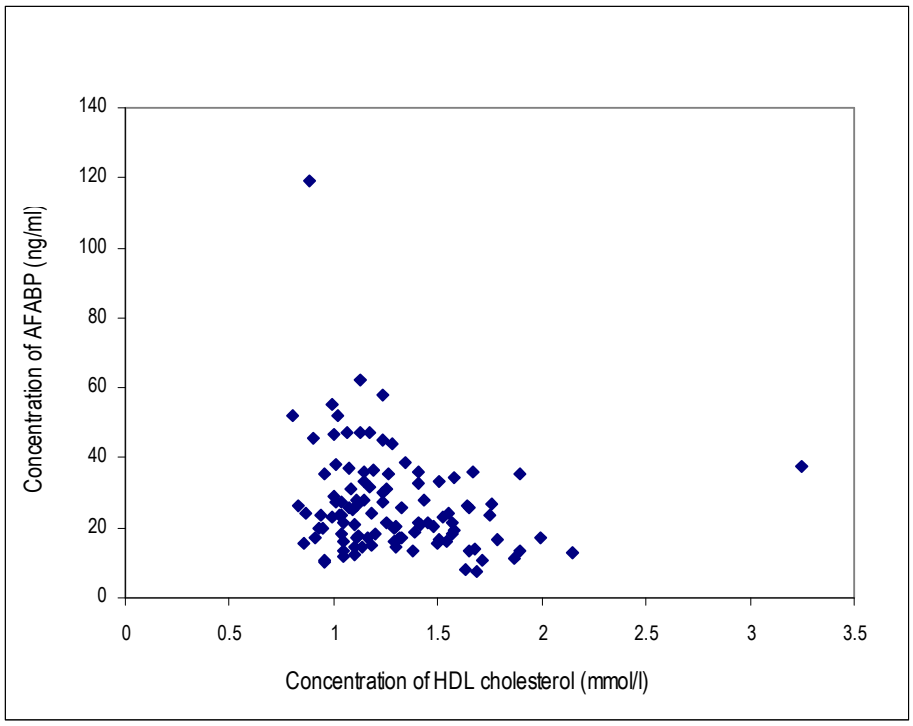


Figure 6: Serum AFABP negatively correlates with HDL.

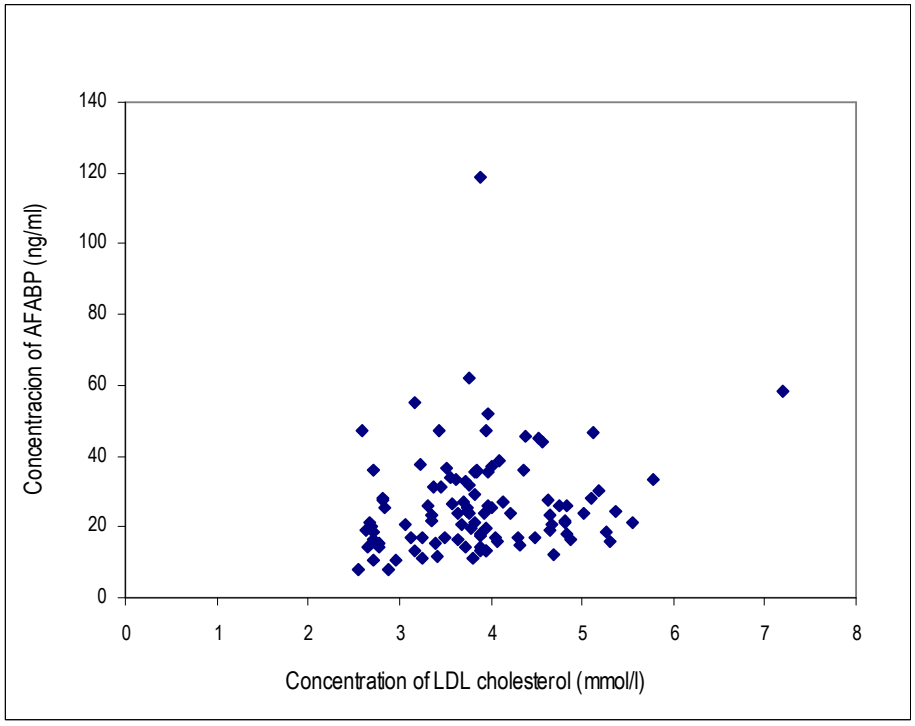


Figure 7: Serum AFABP positively correlates with LDL.

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

16. METHOD COMPARISON

The BioVendor Human AFABP ELISA was not compared to the other commercial immunoassays.

17. 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

18. REFERENCES

References to adipocyte FABP:

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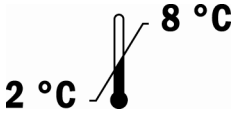
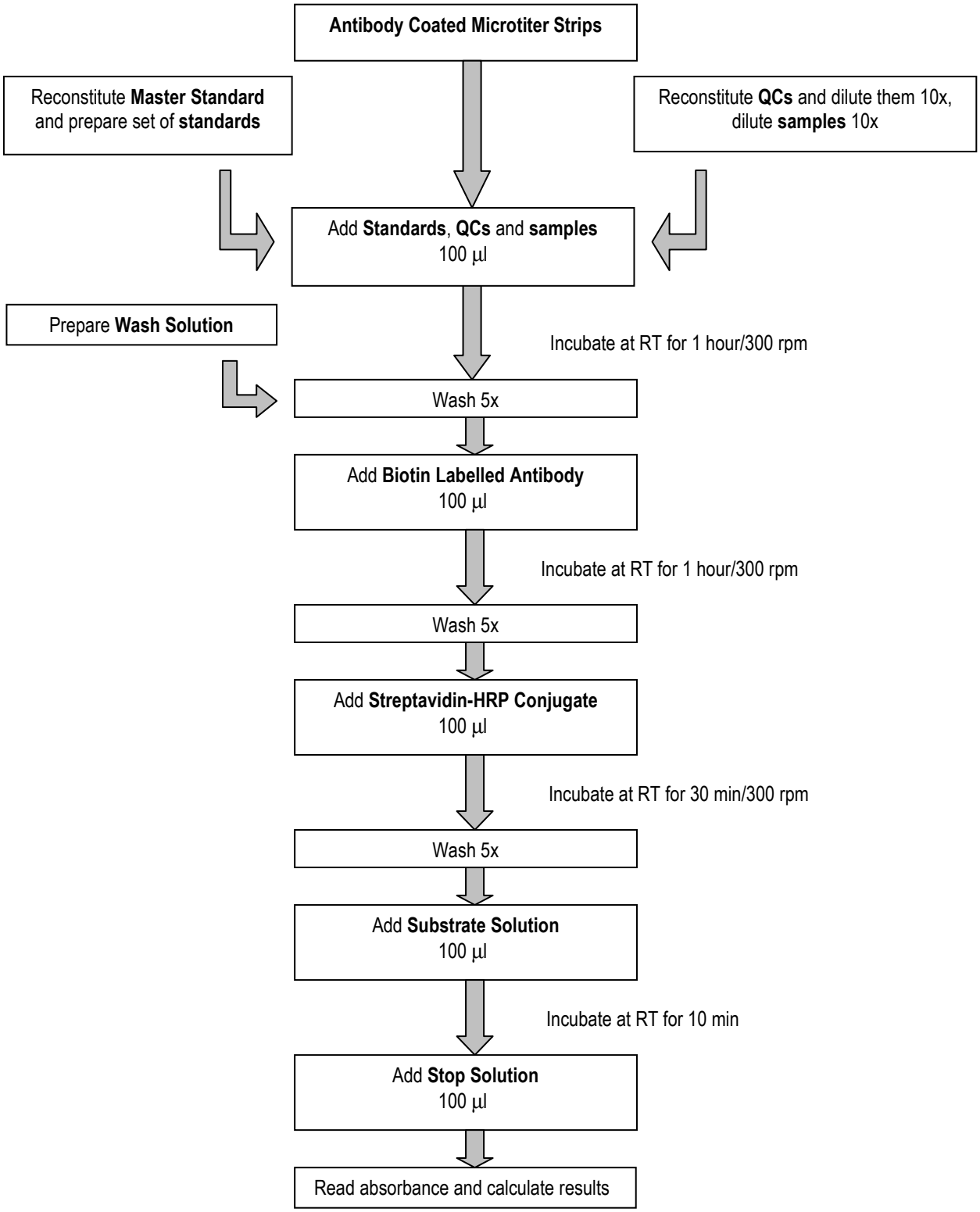
›› For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary



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NOTES

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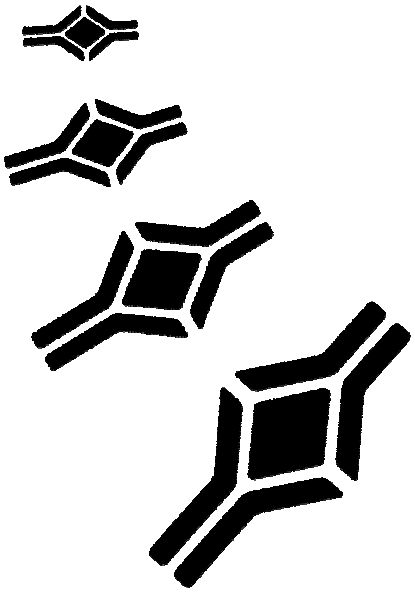
and Diagnostic Products



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