

# Human Adipocyte FABP ELISA (Human FABP4 ELISA)

Cat. No.: RD191036200R

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

## 1. Intended Use

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The RD191036200R AFABP ELISA is a biotin labelled antibody based sandwich enzyme immunoassay for the quantitative measurement of human AFABP in serum, plasma, tissue extracts and tissue culture medium. It is intended for *research use only*.

### Features

- The total assay time is less than four hours.
- The kit measures total serum or plasma AFABP.
- Quality Controls are human serum based. No animal sera are used.
- **Patent Application Number:**  
**DE 10 2005 034 788.6**

## 2. Storage, Expiration

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Store the kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

### 3. Summary

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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 (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<sup>-/-</sup> 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.

#### **4. Test Principle**

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In the BioVendor's Human AFABP ELISA, calibrators or samples are incubated with a goat polyclonal anti-human AFABP antibody coated in microtiter wells. After one-hour incubation and a washing, biotin-labelled polyclonal anti-human AFABP antibody is added and incubated with captured AFABP for one hour. After a thorough wash, streptavidin-horseradish peroxidase conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate H<sub>2</sub>O<sub>2</sub>-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of AFABP. A standard curve is constructed by plotting absorbance values versus AFABP concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

#### **5. Precautions**

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- For *research use only*.
- This kit contains components of human origin. These materials were found non-reactive for hepatitis B surface antigen and for HIV antibody. However, these materials should be handled as potentially infectious, as no tests can guarantee the complete absence of infectious agents.
- Avoid contact with the acidic Stop Solution and Substrate Solution which contains hydrogen peroxide. Wear gloves and eye protection when handling these reagents. In case of contact with the Stop Solution and the Substrate Solution wash skin thoroughly with water and seek medical attention, when necessary.
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- The materials must not be pipetted by mouth.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- Reagents with different lot numbers should not be mixed.
- Reagents should not be used after the expiration specified on the kit label.

## 6. Reagents Supplied

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<i>Cat. No.</i>	<i>Kit Components</i>	<i>Quantity</i>
C281111	Microtiter Strips, coated with capture polyclonal Anti-AFABP Antibody, sealed	96 wells
C282531	Biotin Labelled Anti-AFABP Antibody, ready to use	13 ml
C282341	Streptavidin-HRP Conjugate, ready to use	13 ml
C283141	AFABP Master Calibrator, lyophilized	1 vial
C284151	Quality Control High, lyophilized	1 vial
C284251	Quality Control Low, lyophilized	1 vial
C005111	Dilution Buffer, ready to use	2x13 ml
C006121	Wash Solution Concentrate (10x)	100 ml
C007111	Substrate Solution (TMB), ready to use	13 ml
C008111	Stop Solution (0.2 M H <sub>2</sub> SO <sub>4</sub> ), ready to use	13 ml
-	Instruction Manual + Certificate of Analysis	1 pc

## 7. Materials Required but Not Supplied

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- Test tubes for diluting samples
- Precision pipettes to deliver 10-1000 µl and disposable tips
- Multichannel pipette 100 µl
- Microplate reader with 450 nm filter
- Microplate shaker (optional)
- Software package facilitating data generation and analysis (optional)
- Microtitration plate washer (optional) [Manual washing is possible but not preferable.]
- Glassware (graduated cylinder and bottle for Wash Solution)
- Deionized (distilled) water

## 8. Preparation of Reagents

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All reagents need to be brought to room temperature prior to the assay.

- If you do not use the whole plate, return unused strips in the provided aluminium bag with desiccant and seal the bag carefully. Keep the unused strips at 2-8°C, protected from the moisture.

Assay reagents are supplied ready-to-use, with the exception of AFABP Master Calibrator, Quality Controls and Wash Solution Concentrate (10x). Preparation of reagents for 1 plate:

### Wash Solution

Dilute 100 ml of Wash Solution Concentrate with 900 ml of deionized (distilled) water.

### Stability and storage:

The diluted Wash Solution is stable for one month when stored at 2-8°C.

### Human AFABP Calibrators

**For actual volume of Dilution Buffer for reconstitution refer to the Certificate of Analysis.** Reconstitute the lyophilized Master Calibrator 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 calibrators using Dilution Buffer as follows:

<i>Calibrator volume</i>	<i>Dilution Buffer Volume</i>	<i>Concentration</i>
stock	-----	25 ng/ml
500 µl of stock	750 µl	10 ng/ml
500 µl of std. 10 ng/ml	500 µl	5 ng/ml
500 µl of std. 5 ng/ml	500 µl	2.5 ng/ml
500 µl of std. 2.5 ng/ml	750 µl	1 ng/ml
500 µl of std. 1 ng/ml	500 µl	0.5 ng/ml

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

### Stability and storage:

Calibrators are stable until the expiration date (see label on the box) when stored at -20°C.

## **Quality Controls**

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

Dilute reconstituted Quality Controls prior to use in the assay 1:10 with Dilution Buffer, e.g. 20 µl Quality Control + 180 µl Dilution Buffer when assaying samples in singlets, or preferably 30 µl sample + 270 µl Dilution Buffer for duplicates.

### Stability and storage:

Reconstituted but undiluted Quality Controls are stable until the expiration date (see label on the box) when stored at -20°C.

Do not store the diluted (1:10) Quality Controls.

## **9. Preparation of Samples**

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Dilute serum or plasma samples prior to use 1:10 with Dilution Buffer, e.g. 20 µl sample + 180 µl Dilution Buffer when assaying samples in singlets, or preferably 30 µl sample + 270 µl Dilution Buffer for duplicates.

### Stability and storage:

See chapter 15.

Do not store the diluted (1:10) samples.

Ask for protocol at [info@biovendor.com](mailto:info@biovendor.com) if assaying tissue extracts, urine, breast milk and tissue culture medium samples.

## 10. Assay Procedure

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- 1) Pipet 100  $\mu$ l of diluted Calibrators, 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).
- 4) Pipet 100  $\mu$ l of Biotin Labelled Anti-AFABP Antibody Solution 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).
- 7) Pipet 100  $\mu$ l of Streptavidin-HRP Conjugate.
- 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).
- 10) Pipet 100  $\mu$ l of Substrate Solution. (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). No shaking!
- 12) Stop the colour development by adding 100  $\mu$ l 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 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.*

	strip 1+ 2	strip 3 + 4	strip 5+ 6	strip 7+ 8	strip 9+10	strip 11+ 12
<b>A</b>	Calibrator 25	QC Low	Sample 8	Sample 16	Sample 24	Sample 32
<b>B</b>	Calibrator 10	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
<b>C</b>	Calibrator 5	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
<b>D</b>	Calibrator 2,5	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
<b>E</b>	Calibrator 1	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
<b>F</b>	Calibrator 0,5	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
<b>G</b>	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
<b>H</b>	QC High	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of work sheet.

## 11. Calculations

Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of calibrators versus *log* of the known concentration (X) of calibrators, using the four-parameter function.

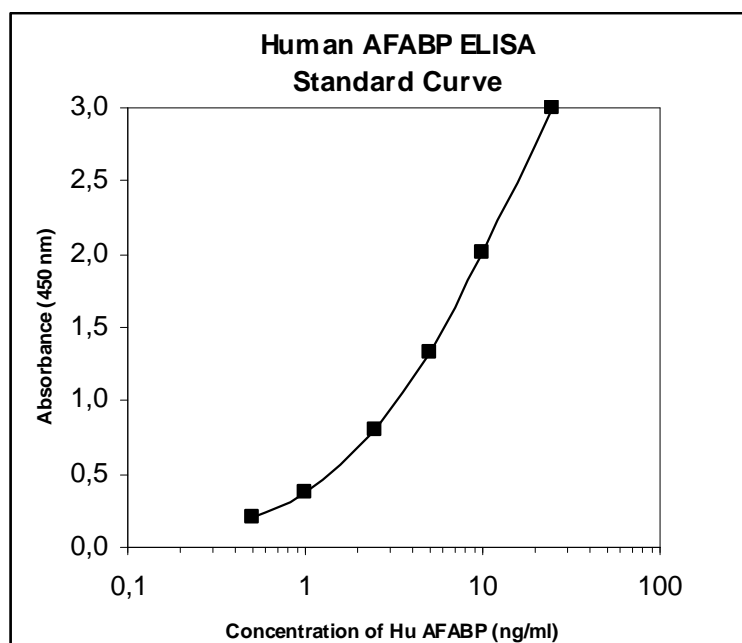


Figure 2: Standard Curve for Human AFABP is plotted using the four-parameter function as a proportion of Human AFABP concentration and absorbance at 450 nm.

Alternatively, the *logit log* function can be used to linearize the calibration curve (i.e. *logit* of absorbance (Y) is plotted versus *log* of the known concentration (X) of calibrators).

**As the Calibrators have not to be diluted, while the samples and the Quality Controls 10-times, the values of samples and Quality Controls calculated from the calibration curve have to be multiplied by a dilution factor of 10 to obtain the true results!**

## **12. Limits of Assay**

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Results exceeding AFABP level of 25 ng/ml as 10-fold diluted should be repeated with greater dilution. Dilution factors need to be taken into consideration in calculating the AFABP concentration.

## **13. Performance Characteristics**

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

For actual Standard curve and Quality Controls values see the Certificate of Analysis.

- **Sensitivity**

The limit of detection (defined as human AFABP concentration giving absorbance higher than mean absorbance of blank\* plus three standard deviations of the absorbance of blank:  $A_{\text{blank}} + 3xSD_{\text{blank}}$ ) is defined as follows:

Analytical Limit of Detection is calculated from the real AFABP values in wells and is 0.1 ng/ml

Assay Sensitivity takes the dilution of samples into consideration and is calculated according to the formula:

Assay Sensitivity = Analytical Limit of Detection x sample dilution = 0.1 ng/ml x 10 = 1ng/ml

\*Dilution Buffer is pipetted into blank wells.

- **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.

No signal was found to the following animal sera: mouse, rabbit, horse, goat, pig, hamster, chicken, rat, sheep and bovine. However, we have found crossreactivity to monkey serum. For details please contact us at [info@biovendor.com](mailto:info@biovendor.com).

- **Precision**

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1	13.9	0.92	6.6
2	27.3	1.08	3.9

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
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 1:10 and assayed.

<i>Sample</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
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 (diluted 1:10 with Dilution Buffer) were further serially diluted with Dilution Buffer (see table below) and assayed.

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

#### **14. Definition of AFABP Master Calibrator**

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A recombinant protein is used as the calibrator. The recombinant AFABP is a 14.7 kDa protein containing 132 amino acid residues.

AFABP concentration depends on the method used for total protein determination. Master calibrator in the kit contains 25 ng of AFABP measured by BCA method (Sigma-Aldrich).

## 15. Preliminary study (unpublished 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.

### 1/ Normal value and normal range in human serum

Group definition: sera taken from 66 random selected women, 35-52 years old

Normal value (mean +/- SEM) = 19.58 +/- 1.01

Normal range (mean +/- 2 SD) = 19.58 +/- 16.32

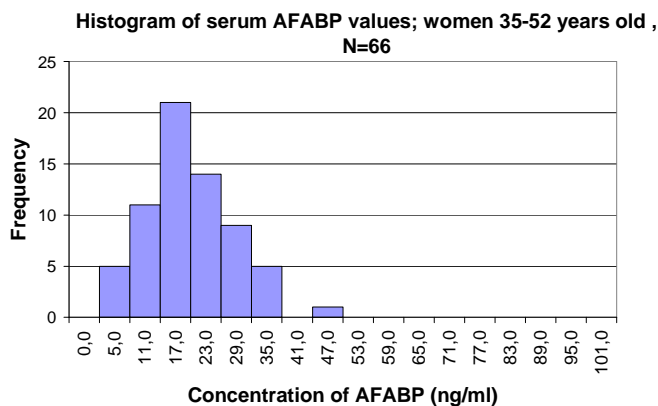


Figure 3: Histogram of serum AFABP values shows Gaussian distribution

### 2/ 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 (ng/ml)
Women	45.5	5.21	1.46	3.35	1.13	21.18
Men	43.7	5.08	1.06	3.70	1.61	21.44

3/ Clinical relevance of serum AFABP value

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

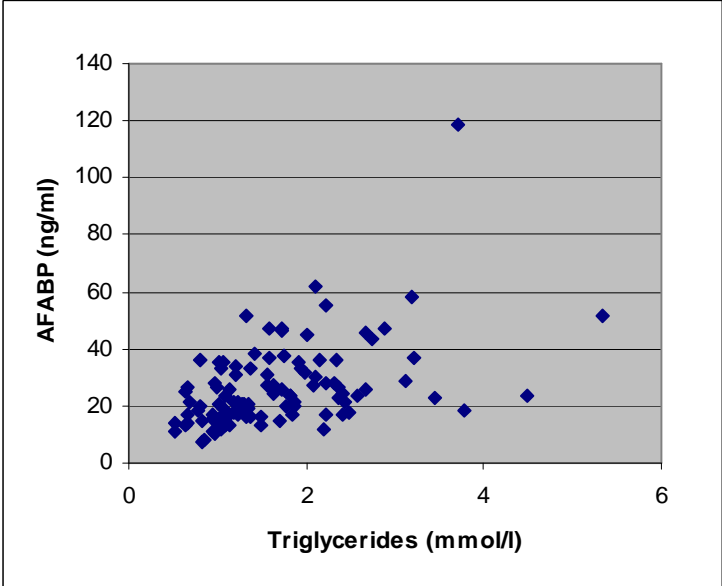


Figure 4: Serum AFABP positively correlates with triglycerides

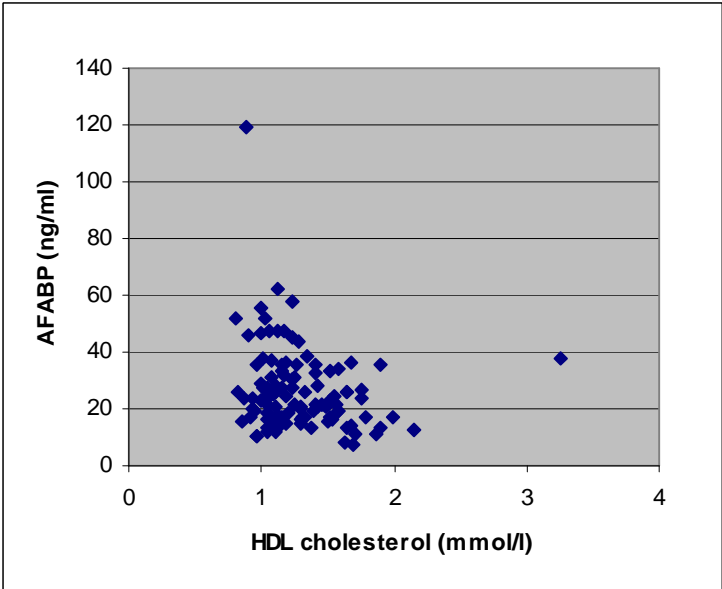


Figure 5: Serum AFABP negatively correlates with HDL

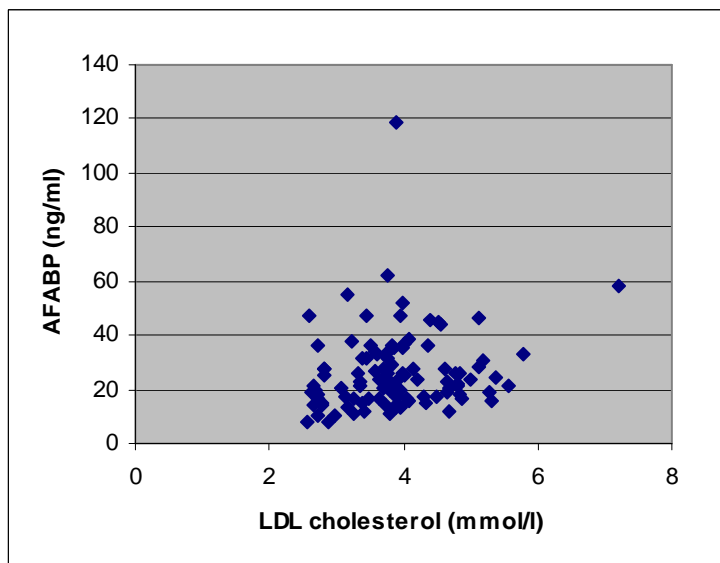


Figure 6: Serum AFABP positively correlates with LDL

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

	Number of volunteers (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

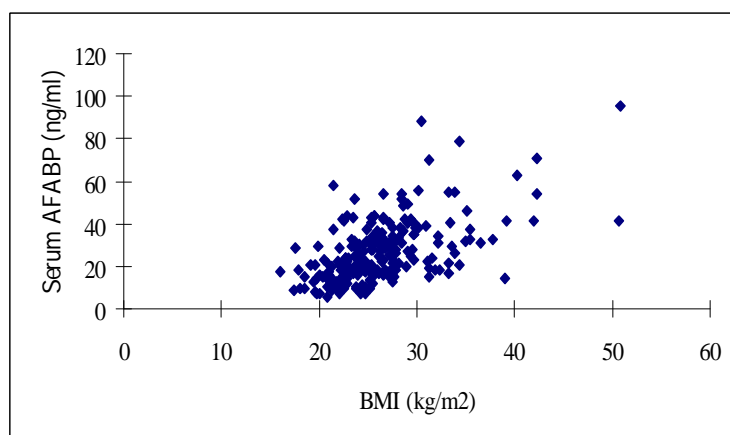


Figure 7: Serum AFABP correlates with BMI

Moreover, serum AFABP is related to insulin sensitivity:

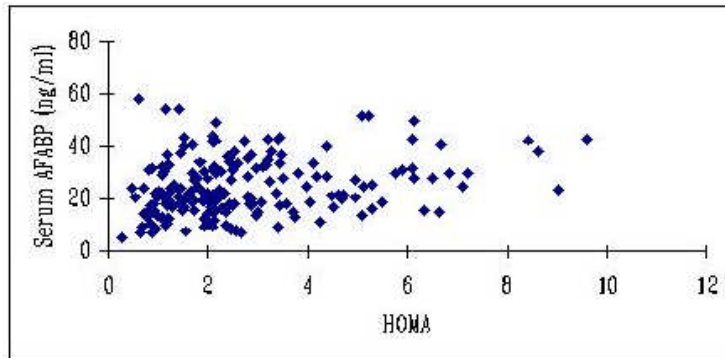


Figure 8: Serum AFABP correlates with HOMA score

## 16. Troubleshooting and FAQs

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### 1/ 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

### 2/ High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time should be decreased before addition of Stop Solution

### 3/ High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing

#### 4/ Effect of freezing/thawing on the concentration of AFABP in samples

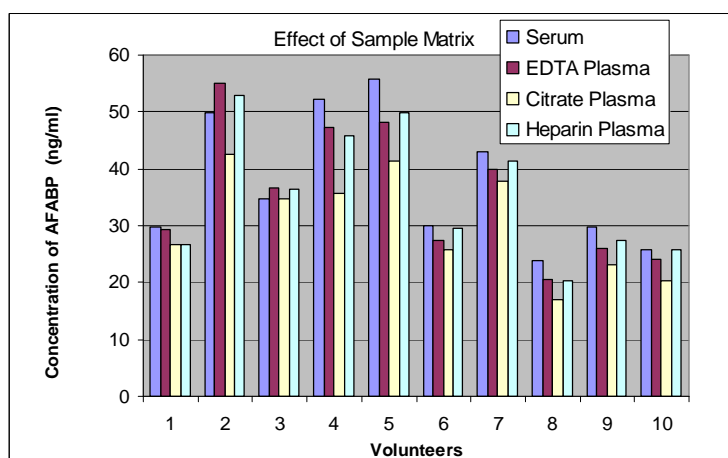
No significant decline was observed in concentration of AFABP in serum or plasma samples after repeated (3x) freezing/thawing cycles.

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

#### 5/ Effect of sample matrix (serum/plasma)

Samples from 10 volunteers were taken and treated by different methods, 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	37.5	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 values of AFABP in serum, citrate plasma, EDTA plasma and heparin plasma:

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

#### **6/ Stability of samples at 4°C**

Samples should be stored at -20°C. However, no decline was observed in concentration of AFABP in serum and plasma samples when stored at 4°C for 2 weeks. To avoid microbial contamination add NaN<sub>3</sub> to a final concentration 0,1% to the samples.

## 17. References

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### References to this product

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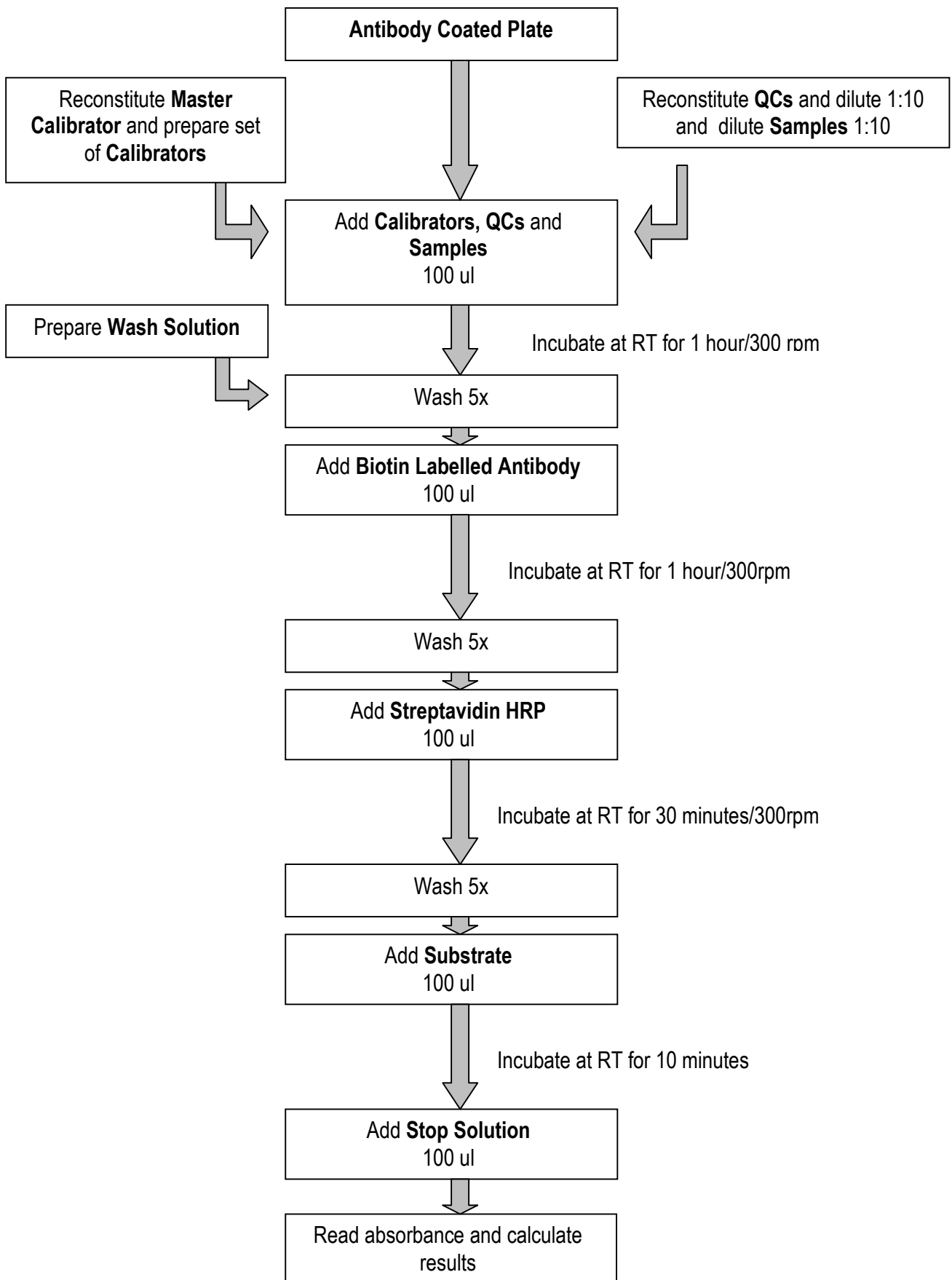
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**Notes:**

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