QUANTITATIVE DETERMINATION OF HUMAN ADIPOCYTE FABP

IVD CE

HUMAN ADIPOCYTE FABP (FABP4) ELISA

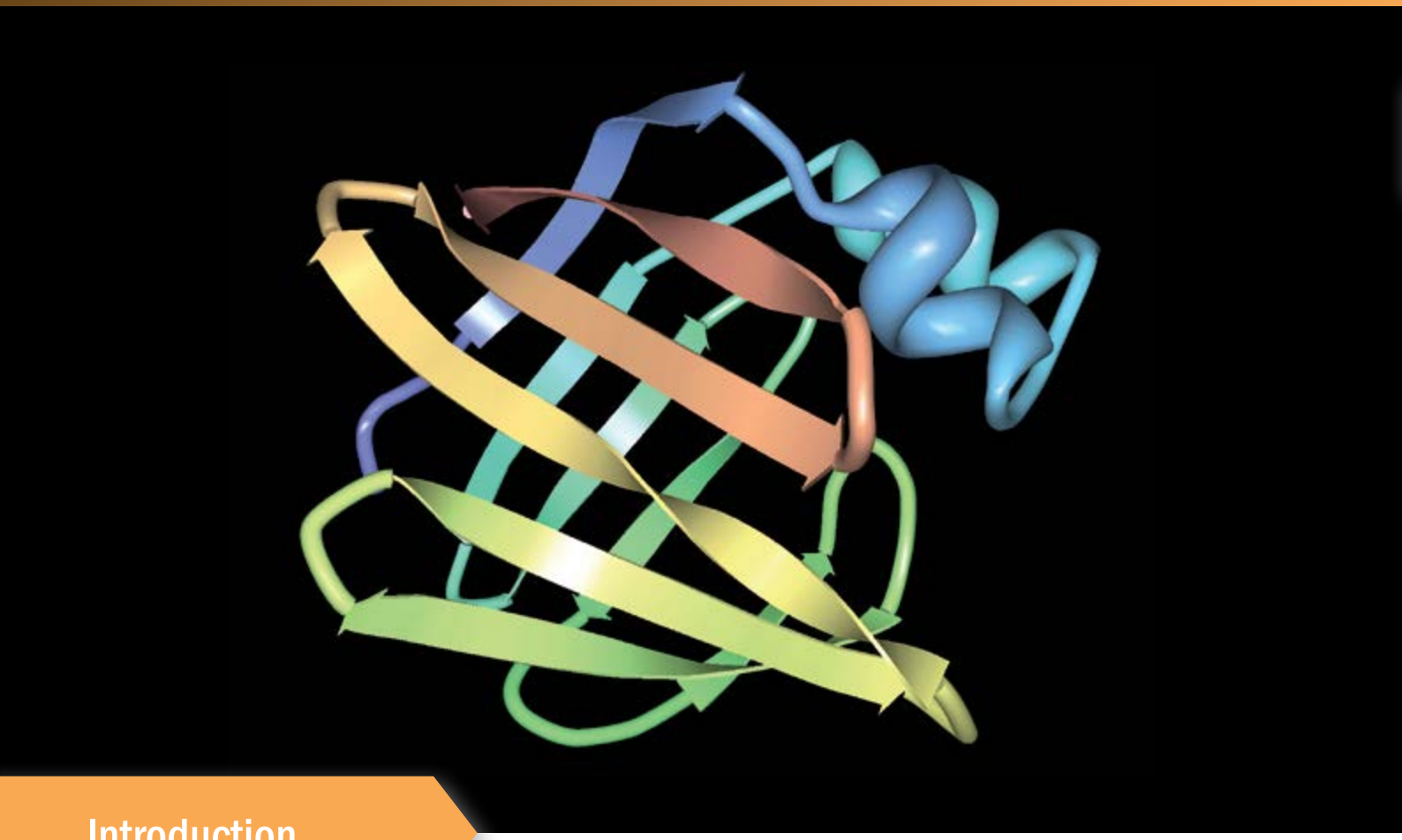
- High sensitivity (0.05 ng/ml)
- Excellent analytical characteristics
- Validated for human plasma (Citrate, EDTA, Heparin), serum

ENERGY METABOLISM AND BODY WEIGHT REGULATION

DIABETOLOGY

BioVendor
Research and Diagnostic Products

HUMAN ADIPOCYTE FABP



Introduction

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.

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BioVendor Human Adipocyte FABP (FABP4) ELISA (RD191036200R)

Intended use

The RD191036200R Human AFABP (FABP4) ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human AFABP.

- **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

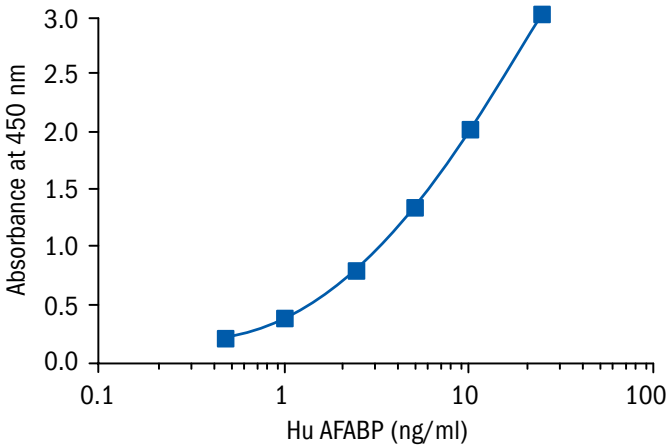
Test principle

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.

Clinical application

- Energy metabolism and body weight regulation
- Diabetology

HUMAN ADIPOCYTE FABP (FABP4) ELISA CAT. NO.: RD191036200R	
Assay format	Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit
Samples	Plasma (Citrate, EDTA, Heparin), Serum
Standards	0.5 to 25 ng/ml
Limit of detection	0.05 ng/ml



Precision

Intra-assay (Within-Run) (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 (Run-to-Run) (n=6)

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

Spiking recovery

Serum samples were spiked with different amounts of human AFABP and assayed.

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

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	59.50	-	-
	2x	29.90	29.75	100.5
	4x	14.41	14.87	96.8
	8x	7.57	7.44	101.7
2	-	52.62	-	-
	2x	26.37	26,31	100.2
	4x	13.01	13.15	98.9
	8x	6.77	6.58	102.9

Effect of sample matrix

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

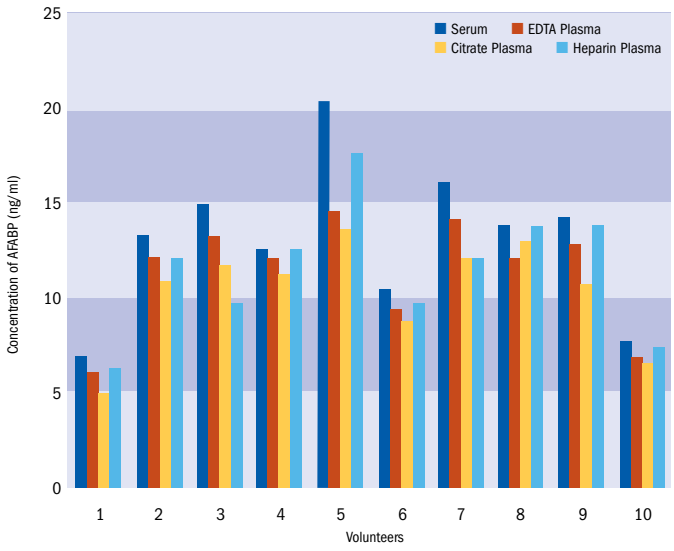


Figure 3: AFABP levels measured using Human AFABP ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

Volunteer No.	Serum (ng/ml)	Plasma (ng/ml)		
		EDTA	Citrate	Heparin
1	6.87	6.05	4.86	6.41
2	13.51	12.18	10.93	12.16
3	14.94	13.28	11.71	9.74
4	12.67	12.04	11.30	12.55
5	20.33	14.53	13.82	17.72
6	10.46	9.43	8.94	9.68
7	16.09	14.21	12.30	12.36
8	13.94	12.30	11.90	13.94
9	14.31	12.90	10.3	13.80
10	7.55	6.92	6.64	7.42
Mean (ng/ml)	**13.07**	**11.38**	**10.33**	**11.58**
Mean Plasma/ Serum (%)	-	**87.1**	**79.1**	**88.6**
Coefficient of determination R^2	-	**0.91**	**0.84**	**0.91**

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Summary of protocol

- Reconstitute Master Standard and prepare set of Standards
- Reconstitute QCs and dilute them 10., dilute samples 10.
- Add 100 µl Standards, QCs and samples
- Incubate at RT for 1 hour/300 rpm
- Wash plate 5 times
- Add 100 µl Biotin Labelled antibody
- Incubate at RT for 1 hour/300 rpm
- Wash plate 5 times
- Add 100 µl Streptavidin-HRP Conjugate
- Incubate at RT for 30 min/300 rpm
- Wash plate 5 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl stop solution
- Read absorbance and calculate results

Related products

- RD291036200R Adipocyte FABP Mouse ELISA
- RD172036100 Adipocyte Fatty Acid Binding Protein Human *E. coli* Tag free
- RD165036050 Adipocyte Fatty Acid Binding Protein NATIVE, Human Adipose Tissue
- RD181037050 Adipocyte Fatty Acid Binding Protein Human, Rabbit Polyclonal Antibody
- RD184037100 Adipocyte Fatty Acid Binding Protein Human, Goat Polyclonal Antibody

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