QUANTITATIVE DETERMINATION OF HUMAN AFAMIN

NEW PRODUCT

Human Afamin ELISA

- Sensitivity (0.022 µg/ml)
- Patent WO2009029971A1: Method for diagnosing the metabolic syndrome
- Validated for human serum and plasma

ENERGY METABOLISM AND BODY WEIGHT REGULATION

BioVendor
Research
and Diagnostic Products

HUMAN AFAMIN ELISA

Introduction

Mature human afamin, the product of the AFM gene, is a single chain 75kDa protein consisting of 578 amino acid residues. It contains three consecutive albumin domains (aa 36-206, 211-403 and 404-599) that contain characteristic 5 or 6 intra-chain disulfide bonds. AFM is a member of the albumin gene family, which is comprised of four genes that localize to chromosome 4 in a tandem arrangement. These four genes encode structurally related serum transport proteins that are known to be evolutionarily related. The glycoprotein afamin is located on chromosome 4q11–q13 in humans [1].

Afamin is predominantly expressed in the liver and secreted into the bloodstream; minor expressions have been described also in human brain [2], heart, kidney, testis and ovary. Afamin has been reported to bind vitamin E, especially α-tocopherol and γ-tocopherol, two of the most important forms of vitamin E, in vitro and in vivo and to possess multiple binding sites for both tocopherol isomers [3,4]. Comparative proteomics has previously identified afamin as a potential biomarker for ovarian cancer [5]. Patients with ovarian cancer displayed significantly decreased plasma concentrations of afamin by comparison to healthy controls [6], these studies were recently extended by showing significant associations between afamin plasma concentrations and clinical outcomes (response to therapy and survival rates) [7].

Furthermore, human plasma afamin was very recently reported to be highly significantly associated with criteria for metabolic syndrome [8]. Data from a prospective study as well as corresponding data from afamin-transgenic mice suggest an active role of afamin in the development of metabolic syndrome [8]. In patients with polycystic ovary syndrome, afamin might serve as a discriminatory predictive parameter of insulin resistance and metabolic syndrome [9].

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BioVendor Human Afamin ELISA (RD194428100R)

Intended use

The RD194428100R Human Afamin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of native human afamin.

- The total assay time is less than 3 hours
- The kit measures total human afamin in serum, plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Standard is a serum-based protein, the standard was calibrated against the primary standard [reference 3, 4 and 10]
- Components of the kit are provided ready to use, concentrated or lyophilized

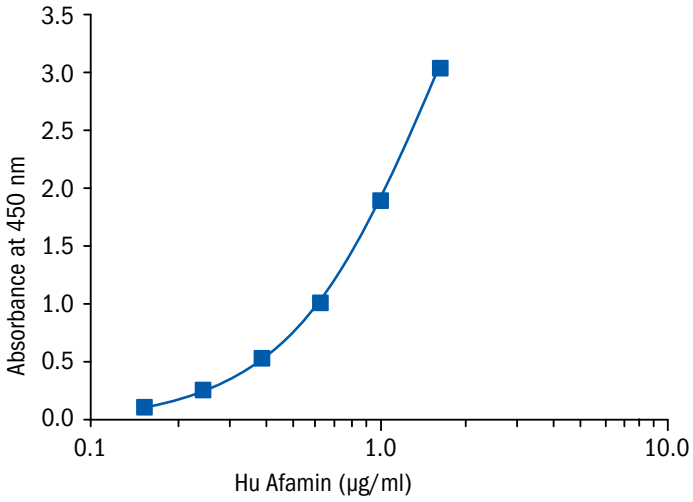
Test principle

In the BioVendor Human Afamin ELISA, standards and samples are incubated in microtitration wells pre-coated with monoclonal anti-human afamin antibody. After 60 minutes incubation and washing, a second, different monoclonal-human afamin antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with the captured afamin. Following another washing step, the remaining HRP 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 afamin. 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

HUMAN AFAMIN ELISA CAT. NO.: RD194428100R	
Assay format	Sandwich ELISA, HRP-labelled antibody, 96 wells/kit
Samples	Serum, Plasma (EDTA, citrate, heparin)
Standards	1.6 – 0.15 µg/ml
Limit of detection	0.022 µg/ml



Precision

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

Sample	Mean (µg/ml)	SD (µg/ml)	CV (%)
1	59.46	1.44	2.43
2	82.92	2.99	3.61

Inter-assay (Run-to-Run) (n=5)

Sample	Mean (µg/ml)	SD (µg/ml)	CV (%)
1	56.03	1.2	2.1
2	79.53	3.4	3.4

Spiking recovery

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

Sample	Observed (µg/ml)	Expected (µg/ml)	Recovery O/E (%)
1	0.361	-	-
	0.552	0.56	108.4
	0.762	0.76	100.1
	1.185	1.16	102.1
2	0.369	-	-
	0.568	0.57	99.8
	0.779	0.77	101.3
	1.186	1.17	101.5

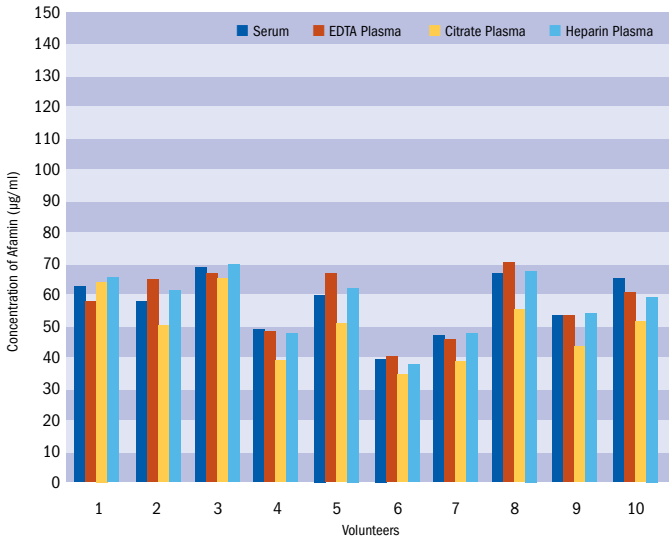
Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (µg/ml)	Expected (µg/ml)	Recovery O/E (%)
1	-	84.40	-	-
	2x	42.00	42.20	99.5
	4x	22.70	21.10	98.1
2	-	89.80	-	-
	2x	45.60	44.90	101.6
	4x	22.70	22.45	101.1

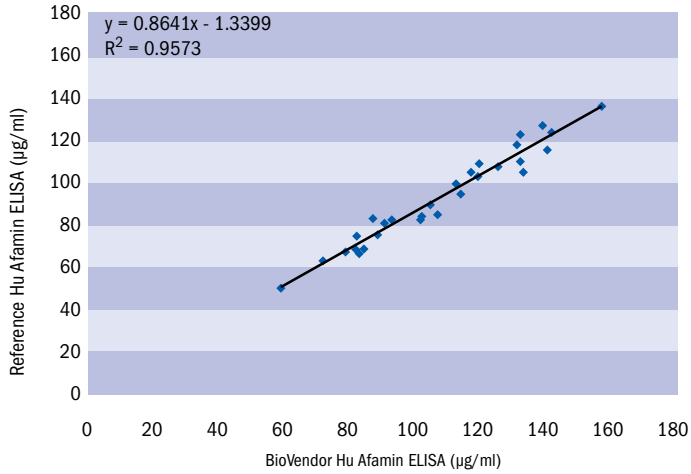
Effect of sample matrix

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



Method Comparison

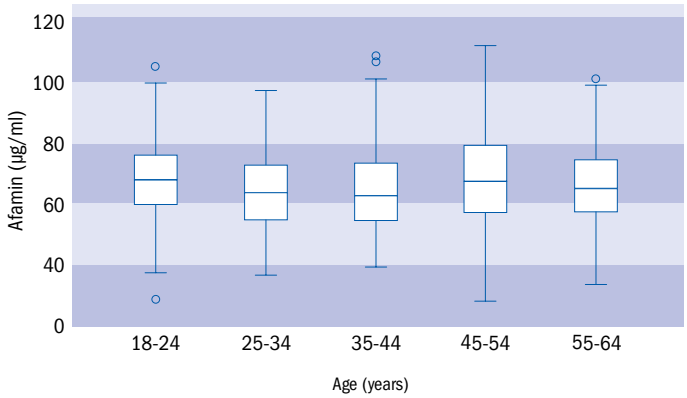
The BioVendor Human Afamin ELISA has been compared to a reference immunoassay (references 3-10) by measuring 29 serum samples. Linear regression analysis of concentration data yielded the following results:



HUMAN AFAMIN ELISA

Population Data

The following results were obtained in EDTA plasma samples from 528 healthy blood donors (338 men + 190 women, 18–64 years old) [data from reference 10]:



Median afamin plasma concentrations according to age groups were as follows:

18–24 years (n = 94), 71 µg/ml (range 33–106 µg/ml);
25–34 years (n = 131), 66 µg/ml (range 40–98 µg/ml);
35–44 years (n = 128), 66 µg/ml (range 43–109 µg/ml);
45–54 years (n = 127), 70 µg/ml (range 33–113 µg/ml);
55–64 years (n = 48), 68 µg/ml (range 38–102 µg/ml)

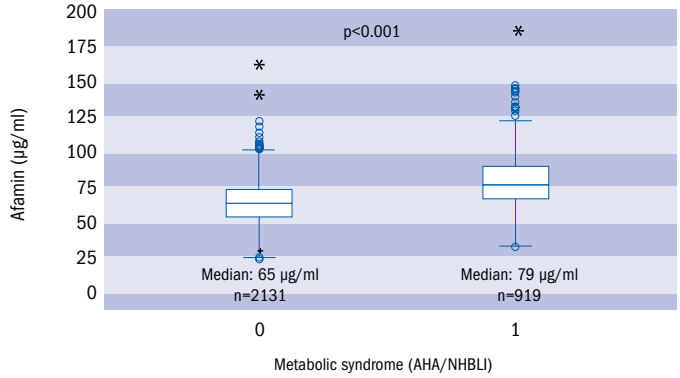
Summary of protocol

- Reconstitute Master Standard and prepare set of Standards
- Dilute samples (serum 100x)
- Add 100 µl Standards and samples
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Conjugate Solution (HRP conjugate)
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl Stop Solution
- Read absorbance and calculate results

Clinical Relevance

In the first large population-based study of 3 independent populations with more than 5,000 participants, a highly significant association between afamin concentrations and the prevalence and development of metabolic syndrome and all its components could be demonstrated (data from reference [8]):

Serum afamin concentrations in individuals without the metabolic syndrome (0) (N=2131, median: 65 µg/ml) and individuals with metabolic syndrome (1) (N=919, median: 79 µg/ml, p<0.001).



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