Vaspin
ELISA E106

Vaspin (Visceral adipose tissue-derived serpin; SerpinA12) is serine protease inhibitor, no target protease is known until today. Vaspin is synthesized in visceral adipose tissue but also in skin, hypothalamus, pancreatic islets and stomach. Vaspin consists of 395 amino acids forming 3 β-sheets and 9 α-helices. Molecular weight of Vaspin is about 45.2 kDa. It does not form multimeric aggregates or intra-molecular disulfide bridges and no binding proteins in human serum are known. The Vaspin gene is not only expressed by subcutaneous and visceral adipose but also by liver tissue, in the pancreas and in the human epidermis (granular keratinocytes / GK cells).

Vaspin shows significant food-intake dependent diurnal rhythms. The nadir is usually in the mid-afternoon and the highest concentration is measured at nighttime with about 250% of the nadir.

The diagnostic value of Vaspin remains unclear, conflicting results question its value as biomarker for visceral or total adipose tissue. As well as regarding insulin resistance while in children Vaspin might correlate with insulin sensitivity but in adults no correlation was found.

The Mediagnost Vaspin ELISA is a tool for the investigation and validation of Vaspin as a biomarker for the visceral adipose tissue, insulin sensitivity and glucose tolerance.

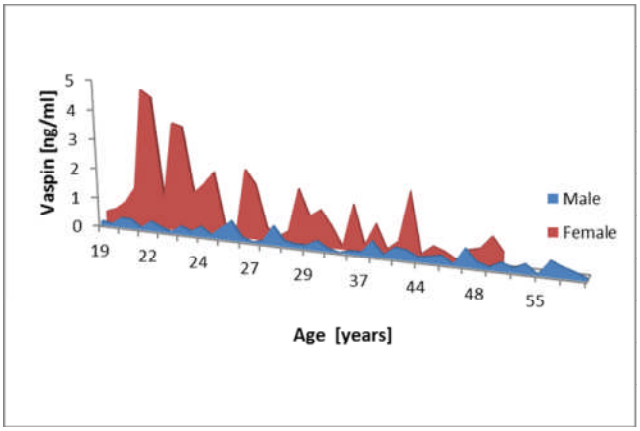

Figure 1 Vaspin concentration in healthy male and female humans of different age.

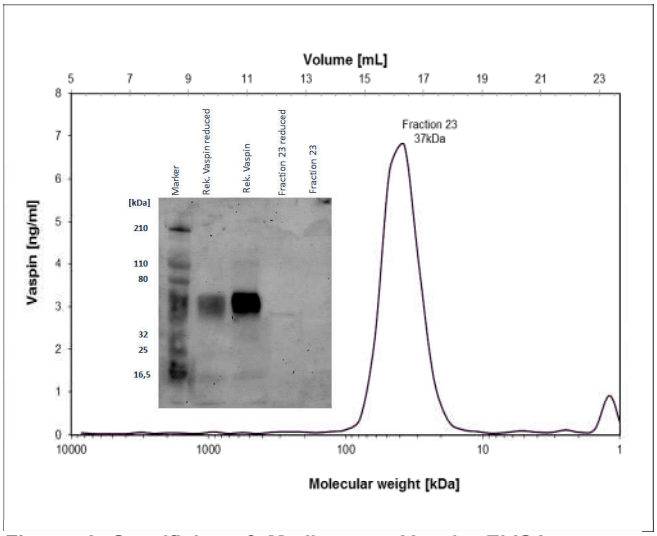

Figure 2 Specificity of Mediagnost Vaspin ELISA. Human Serum (250 µL) was separated by SEC Superdex 10/300GL, fraction size was 0.5 mL and flow velocity 0.5mL/min. Samples were diluted 1:4 in dilution buffer. Inset shows results of western blotting, samples were separated by 10% SDS-PAGE blotted and stained by biotinylated antibody (1:1000) and streptavidin-peroxidase conjugate (1:1500).

Assay Characteristics

Mediagnost Vaspin ELISA E106

The Mediagnost Vaspin ELISA E106 is based on polyclonal antibodies, raised by genetic immunization using the sequence Vaspin SC306941.

Specificity was proved by western blotting and size-exclusion chromatography (Fig. 2).

- ✓ Standard Range 10 – 1000 ng/L
- ✓ Analytical Sensitivity (LoB) 4 ng/L
- ✓ Recovery of 50 ng/L was between 88 -118% in 18 serum samples
- ✓ Linearity of 2 serum samples was excellent 1:2 - 1:32
- ✓ Correlation with commercial test system: R^2=0.98

The test system is intended: For Research Use Only. Not for use in diagnostic procedures.

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Mediagnost VASPIN ELISA E106

Reconstitution / Dilution of Reagents		
Standards A-E	Reconstitution in **Dilution Buffer VP**	**750 µl each**
Control Serum KS1 & KS2	Reconstitution in **Dilution Buffer VP**	**250 µl each**
Washing Buffer WP	dilute in **A. dest.** (e.g. add the complete contents of the flask 50 ml into a graduated flask and fill with A.dest. to 1000 ml)	**1:20**
Sample Dilution + Control Sera KS1 & KS2: 1:4 in Dilution Buffer VP, **mix directly and use within max. 60 min.** Use **100 µl per determination**		
Before assay procedure bring all **reagents** to **room temperature**		

Proposal of Assay Procedure for Double Determination:

Pipette	Reagents	Well Positions
100 µl	Blank (**VP**)	A1 and A2
100 µl	Standard **A** (**10 pg/ml**)	B1 and B2
100 µl	Standard **B** (**75 pg/ml**)	C1 and C2
100 µl	Standard **C** (**200 pg/ml**)	D1 and D2
100 µl	Standard **D** (**500 pg/ml**)	E1 and E2
100 µl	Standard **E** (**1000 pg/ml**)	F1 and F2
100 µl	Diluted Control Serum **KS1 (1:4)**	G1 and G2
100 µl	Diluted Control Serum **KS2 (1:4)**	H1 and H2
100 µl	**Diluted Samples (1:4)**	Pipette sample in the rest of the wells according to requirements
Cover the wells with the sealing tape		

Incubation: 1 h at RT (20-25°C), 350 rpm

5 x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP/well**	each well
100 µl	Antibody- Conjugate **AK**	each well

Incubation: 1 h at RT (20-25°C), 350 rpm

5 x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP/well**	each well
100 µl	Enzyme-Conjugate **EK**	each well

Incubation: 1 h at RT (20-25°C), 350 rpm

5 x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP/well**	each well
100 µl	Substrate Solution **S**	each well

Incubation: 0.5 h in the dark at RT (20-25°C)

100 µl	Stop Solution **SL**	each well
Measure the absorbance within 30 min at **450 nm** (≥590 nm Reference)		