Human Resistin
ELISA E50

Resistin, a cysteine-rich protein of 11,3 kDa, was firstly found in mice and constitutes together with RELMα, RELMβ and RELMγ the protein family of **resistin-like molecules** (RELM).

In humans, Resistin and RELMβ but no other proteins of the RELM family were found. The human form of Resistin shows a homology of 53% to the murine protein. It has 11 cysteine-residues, is synthesized as a propetide of 108 amino acids and secreted as a dimer, build by a disulfide bridge of cysteine residues. Beside this intermolecular disulfide bridge, 5 additional intramolecular ones exist. Appearance of multi- and oligomer formation was proved by size exclusion chromatography.

In humans, **Resistin expression** in adipocytes can be detected but only at a very low level. Human Resistin gene is expressed in **pancreatic islets, pre-adipocytes, macrophages** and **bone marrow**. So, Resistin is of relevance for inflammation processes as well as for lipid metabolism.

Resistin	
Influencing factors	Sex, Puberty, Obesity
Circadian variation	none
Half-Life in Circulation	5 h
Bioactive/Multimeric/ Analog Forms	Homodimeric protein, subunits linked by disulfid bridge Homologs: RELM α (FIZZ1); RELM β (FIZZ2)
Binding Proteins	Not known
Recommended sample matrix	Serum or Plasma
Recommended Sampling time	all day
Sample Stability	
Short term	Serum samples: 3 days at 37°C
Long term	Serum samples 2.5 years at -20°C
Freeze-thaw cycles	3

In human studies results are not clear – several studies show an association of Resistin serum concentration and adiposity or insulin resistance. But others failed in confirming these results. Therefore, there is requirement for **valid and reproducible determination of Resistin** serum concentration.

Experiments with endothelial cells gave interesting results. Here, **Resistin** was shown to **enhance expression of VCAM-1 and ICAM-1**. By this way, Resistin is potentially able to influence **endothelial inflammation** and, thereby atheriosclerosis. These results were confirmed by experiments in mice, where endothelin-1 was shown to regulate Resistin secretion.

Female				**Resistin (ng/ml):**	
Age (Years):	**n:**	**AV Age:**	**AV BMI:**	**AV ± SD:**	**25.- 75. Percentile**
18 - 30	96	23.0	23,1	**7.2** ± 2.6	5.4 – 8.8
31 - 40	63	36.5	24,3	**8.1** ± 2.3	6.4 – 9.6
41 - 50	67	44.9	24,8	**7.3** ± 2.5	5.7 – 8.1
51 - 60	29	54.7	25,0	**7.2** ± 2.6	5.4 – 8.5
61 - 65	9	62.7	25,2	**6.6** ± 1.1	6.0 – 6.7
Male				**Resistin (ng/ml):**	
Age (Years):	**n:**	**AV Age:**	**AV BMI:**	**AV ± SD:**	**25.- 75. Percentile**
18 - 30	107	23.9	24.1	**6,4** ± 1,8	5.0 – 7.6
31 - 40	59	35.9	25.0	**6,7** ± 3,2	4.8 – 7.4
41 - 50	66	45.0	25.2	**6,5** ± 2,8	4.5 – 7.4
51 - 60	36	54.8	26.4	**6,1** ± 2,1	4.7 – 7.2
61 - 68	20	63.2	25.6	**7,2** ± 1,8	6.0 – 8.2

n=Number of Probands,**AV**=Average Value,**BMI**=Body Mass Index (kg/m²),**SD**=Standard Deviation

In recent research human Resistin was shown to increase pre-adipocyte proliferation and lipolysis of mature adipocytes.

Mediagnost Resistin ELISA E50

Highly specific antibodies allow quantitative detection of human Resistin in serum and plasma. Haemolytic samples however are measured non-correctly high. **This ELISA can also be used for Resistin measurement.** In comparison of human and rat/mice probes Mediagnost ELISA recognizes rat/mice Resistin to a high degree. Exact validation is in process.

This assay is calibrated with **recombinant resistin.** We provide reference values of 550 healthy humans measured by Prof. Dr. J. Kratzsch, Institute for Laboratory Medicine, University of Leipzig.

- ✓ Analytical sensitivity of 0.012 ng/ml
- ✓ Results on your hands in about 4 h
- ✓ single standards (0.02, 0.1, 0.3, 0.6 and 1 ng/ml), freeze-dried
- ✓ Intra- and Inter-assay variance ≤ 7%
- ✓ 2 internal control sera: RiliBÄK conform
- ✓ Calibrated against recombinant Resistin

Mediagnost ELISA E50 has an **European Approval for Clinical Diagnostics**:EC Directive 98/79EG (CE).

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MEDIAGNOST RESISTIN ELISA E50

Reagent preparation:	**Reconstitution:**	**Dilution:**
Standards A – E	in **750 µl** Sample Buffer **PP**	
Control Serum KS1	in **250 µl** Dilution Buffer **VP**	**1:21** with Sample Buffer **PP**
Control Serum KS2	in **250 µl** Dilution Buffer **VP**	**1:21** with Sample Buffer **PP**
Antibody Conjugate AK		**1:100** with Dilution Buffer **VP**
Enzyme Conjugate EK		**1:100** with Dilution Buffer **VP**
Washing Buffer WP		**1:20** with **Aqua.dest.** (e.g., add the complete contents of the flask **(50 ml)** into a graduated flask and fill with A.dest. to 1000 ml).
Sample dilution: **1:21** (e.g. 15 µl Serum with 300 µl Sample Buffer **PP)**		

Assay Procedure for Double Determination

Pipette	**Reagents**	**Position**
100 µl	Sample Puffer **PP** (blank value)	A1/2
100 µl	Standard **A** (0.02 ng/ml)	B1/2
100 µl	Standard **B** (0.1 ng/ml)	C1/2
100 µl	Standard **C** (0.3 ng/ml)	D1/2
100 µl	Standard **D** (0.6 ng/ml)	E1/2
100 µl	Standard **E** (1.0 ng/ml)	F1/2
100 µl	Control Serum **KS1** (diluted)	G1/2
100 µl	Control Serum **KS2** (diluted)	H1/2
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
Incubation: 2 h at **RT, 350 rpm**		
5x 300 µl	Aspirate the contents of the wells and wash 5x with **300 µl** Wash Buffer **WP**	each well
100 µl	**1:100** diluted Antibody Conjugate **AK**	each well
Incubation: 1 h at **RT, 350 rpm**		
5x 300 µl	Aspirate the contents of the wells and wash 5x with **300 µl** Wash Buffer **WP**	each well
100 µl	**1:100** diluted Enzyme Conjugate **EK**	each well
Incubation: 30 min at **RT, 350 rpm**		
5x 300 µl	Aspirate the contents of the wells and wash 5x with **300 µl** Wash Buffer **WP**	each well
100 µl	Substrate Solution **S**	each well
Incubation: 30 min in the dark at **RT**		
100 µl	Stop Solution **SL**	each well
Measure the absorbance within 30 min **at** 450 nm **with** ≥ 590 nm **as reference wavelength**		

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