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Gesellschaft für Forschung und Herstellung von Diagnostika GmbH
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Human Adiponectin ELISA E09

Adiponectin is a 30 kDa protein mainly synthesized by adipocytes but in minor quantities also by muscle cells and hepatocytes. Only IGF-I is known as natural inductor of adiponectin synthesis. Different multimeric forms, a high molecular weight form of >300kDa, a hexameric form of about 168kDa and a trimer of 84kDa, can be found by gel filtration analysis in human serum. The biological effect of adiponectin seems to be dependent on the multimeric form. Mediagnost ELISA E09 measures total adiponectin, as proved by gel filtration. Adiponectin is involved in a **broad range of physiological processes**. Beside influencing atherosclerosis and coronary diseases, it exerts influence on glucose – and lipometabolism.

Adiponectin	
Influencing factors	BMI, Sex,
Circadian variation	negligible minor diurnal and post-prandial variations
Half-Life in Circulation	Trimer (17.5); HMW (13h); measured in rabbit, isoform dependent!
Bioactive/Multimeric/ Analog Forms	3 multimeric forms Trimer (84kDa); Hexamer (168kDa); HMW (>336kDa; 12-18mers)
Binding Proteins	not known
Recommended sample matrix	Serum or Plasma
Recommended Sampling time	all day
Sample Stability	
Short term	EDTA-Plasma and Heparin Plasma at 4°C (blue ice): 36h Serum at 37C: 72h
Long term	Plasma and Serum: 2 years at -20°C
Freeze-thaw cycles	not more than 8

Plasma levels are elevated in patients with metabolic syndrome, therefore adiponectin may serve as an **early diagnostic marker** to identify patients with insulin resistance. For your convenience we provide **age and gender specific reference values**.

Normal Adiponectin Concentration?

	Female Adiponectin [µg/ml]			Male Adiponectin [µg/ml]		
Age (Years):	**n:**	**BMI: AV ± SD**	**AV ± SD**	**n:**	**BMI: AV ± SD**	**AV ± SD:**
Newborn	19		**29.80** ± 12.49	10		**27.80** ± 7.68
< 3.99	9	15.73 ± 0.79	**14.43** ± 7.76	14	16.17 ± 1.81	**16.57** ± 6.55
4.0-7.99	11	16.01 ± 1.94	**8.46** ± 4.73	12	15.69 ± 1.05	**11.24** ± 5.43
8.0-9.99	22	17.58 ± 3.84	**7.92** ± 3.00	18	16.45 ± 1.76	**8.11** ± 2.93
10.0-11.99	33	17.83 ± 1.86	**7.66** ± 4.59	21	18.34 ± 2.18	**8.43** ± 3.91
12.0-13.99	11	19.85 ± 2.31	**8.22** ± 5.64	14	18.61 ± 2.11	**7.59** ± 2.86
14.0-15.99	27	19.91 ± 1.72	**8.83** ± 9.25	32	19.86 ± 2.00	**7.53** ± 2.52
16.0-19,99	18	21.64 ± 2.64	**9.00** ± 3.22	23	22.03 ± 2.42	**7.16** ± 3.53
20.0-29.99	24	23.12 ± 5.01	**7.39** ± 3.35	23	23.43 ± 2.48	**5.44** ± 2.29
30.0-39.99	17	23.20 ± 2.86	**9.19** ± 3.89	21	23.33 ± 2.72	**5.92** ± 4.60
40.0-49.99	26	24.50 ± 4.11	**9.93** ± 3.59	22	23.79 ± 2.41	**6.13** ± 2.92
50.0-59.99	21	24.61 ± 3.31	**11.5** ± 5.49	23	26.68 ± 2.77	**7.45** ± 4.50
>60.0	8	24.63 ± 1.89	**15.6** ± 4.64	24	25.72 ± 2.12	**7.48** ± 3.92

BMI = body mass index, AV = mean, SD= standard deviation

Even if every laboratory has to establish its own normal values, we provide **reference values**. Adiponectin serum concentration was investigated in 503 healthy humans. Age and sex dependent results can be found in the table above. Median concentrations as well as 5th and 95th percentiles are shown in our product brochure.

Assay Features E09

Mediagnost Adiponectin ELISA E09 allows easy quantification of human adiponectin in different matrices as human serum, plasma or cell culture supernatants. This assay is validated against **recombinant adiponectin** Please see workflow chart overleaf.

- ✓ Analytical sensitivity of 0.6 ng/ml
- ✓ Results on your hands in about 2 h
- ✓ 5 freeze-dried single standards: native human adiponectin (2, 10, 30, 70, 100 ng/ml)
- ✓ 2 internal control sera: RiliBÄK conform
- ✓ Breakable microtiterplates for individual usage

Mediagnost ELISA E09 has an **European Approval for Clinical Diagnostics**: EC Directive 98/79EG (CE)

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Mediagnost Adiponectin ELISA E09

Reagent preparation:	Reconstitution:	Dilution
Standards A-E	in 750 µl Dilution Buffer **VP**	
Control Sera KS1 & KS2	in 500 µl Dilution Buffer **VP**	**1:310 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: Pipette for example 300 µl Dilution Buffer VP in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 10 µl Serum- or Plasma (dilution: 1:31). Add **900 µl** Dilution Buffer **VP** in an other PE-/PP-tube and **100 µl** of the thoroughly mixed first dilution. After mixing, use **2×100 µl** from this **1:310** diluted sample in the assay.		

Pipette	Reagents	Position
100 µl	Dilution Buffer **VP**	A1/2
100 µl	Standard **A** (**2 ng/ml**)	B1/2
100 µl	Standard **B** (**10 ng/ml**)	C1/2
100 µl	Standard **C** (**30 ng/ml**)	D1/2
100 µl	Standard **D** (**70 ng/ml**)	E1/2
100 µl	Standard **E** (**100 ng/ml**)	F1/2
100 µl	Control Serum **KS1**	G1/2
100 µl	Control Serum **KS2**	H1/2
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
Incubation: 1 h at RT, 350 rpm		
3x 300 µl	Aspirate the contents of the wells and wash 3x with **300 µl** Wash Buffer **WP**	each well
100 µl	Antibody-POD-Conjugate **AK**	each well
Incubation: 30 min at RT, 350 rpm		
3x 300 µl	Aspirate the contents of the wells and wash 3x with **300 µl** Wash Buffer **WP**	each well
100 µl	Substrate Solution **S**	each well
Incubation: 15 min in the **Dark** at **RT**		
100 µl	Stopping Solution **SL**	each well
Measure the absorbance within **30 min** at **450 nm** with **≥590 nm** as reference wavelength.		

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