

# Mouse- / Rat-Adiponectin ELISA E091-M / E091-R

Adiponectin also called Acrp30 is one of the most abundant proteins in the circulation. This protein exists in different multimeric forms: Trimer (84 kDa); Hexamer (168 kDa) and a high-molecular weight form a 12-18mer (>400 kDa).

Half Life of human recombinant Adiponectin in plasma of mice/rats is about 7.4 h (Shiabata R et al, 2007), the trimer and high molecular weight forms have significantly increased half-lives of 17.5 and 13 hours in rabbits (Peake PW et al, 2005).

Adiponectin seems to be involved in energy metabolism and inflammatory processes. In coronary disease, atherosclerosis, insulin resistance and obesity decreased adiponectin levels have been reported. Thus, Adiponectin could be of diagnostic relevance in every day diagnostics and of interest as surrogate marker in clinical studies.



The Mediagnost ELISA for mouse/rat Adiponectin are a so-called Sandwich-Assays, for measurement of murine Adiponectin in **Serum/Plasma** Samples as well as in **Cell Culture Supernatants**. The assay is based on a combination of a highly specific monoclonal and a polyclonal antibody. Total Adiponectin is measured by these assays.

Reference Values	
Animal Strain	Adiponectin [µg/mL]
Sprague Dawley rat	18 - 84
PD/Cub rats	1.0 – 5.0
SHR/NAad rats	10.0
C57 BL/6 mice	8.0 12.0
Virgin mice	Up to 56.8

### Specimen

Serum and plasma samples of mice/rats can be used in this assay.

No influence of Heparin (30 IE/mL), EDTA (6.8 mM) and NaCitrat (0.015 M) on the measurement of Adiponectin has been detected by recovery experiments.

### Stability of the Samples

Storage at 25°C up to 2 days

Storage at -20°C up to 2 years

Not more than 3 freeze/thaw cycles!

Linearity Mouse Adiponectin E091-M				
Dilution	Sample 1	Dilution	Sample 2	Sample 3
1:14000	11.673	1:10000	9.59	
1:28000	10.928	1:12000	11.229	11.886
1:56000	10.67	1:24000	9.856	10.822
1:112000	10.421	1:48000	9.91	10.222
		1:96000	9.684	9.999

### Assay Characteristics

#### Mediagnost MOUSE-Adiponectin E091-M

- ✓ Mouse Adiponectin Standard: 0.025 - 1 ng/mL
- ✓ Analytical sensitivity of 0.008 ng/mL
- ✓ High Precision:
  - Inter-Assay Variance of 7.3%
  - Intra-Assay Variance of 2.16%
- ✓ 2 Control Sera for GLP conformity
- ✓ Fast: Incubation Time of 2.5 hours
- ✓ Low sample volume needed:
  - Dilution of 1:10000 recommended!

### Assay Characteristics

#### Mediagnost Rat-Adiponectin E091-R

- ✓ Rat Adiponectin Standard 0.25 - 10 ng/mL
- ✓ Analytical sensitivity of 0.081 ng/mL
- ✓ High Precision:
  - Inter-Assay Variance of 5.14%
  - Intra-Assay Variance of 2.5%
- ✓ 2 Control Sera for GLP conformity
- ✓ Fast: Incubation Time of 2.5 hours
- ✓ Low sample volume needed:
  - Dilution of 1:1500 recommended!

## Mediagnost Mouse Adiponectin ELISA E091-M

Reagent preparation:	Reconstitution:	Dilution
<b>Standards A-F</b>	in 1 ml Dilution Buffer <b>VP</b>	
<b>Control Sera KS1 &amp; KS2</b>	in 250 µl Dilution Buffer <b>VP</b>	<b>1:10 000 with Dilution Buffer VP</b>
<b>Washing Buffer WP</b>		<b>1:20 with Aqua. dest.</b> (e.g., add the complete contents of the flask ( <b>50 ml</b> ) into a graduated flask and fill with A.dest. to 1000 ml).
<b>Sample Dilution: e.g. 1 :10 000</b>		
Pipette	Reagents	Position
100 µl	Dilution Buffer <b>VP</b>	A1/2
100 µl	Standard <b>A ( 0.025 ng/ml)</b>	B1/2
100 µl	Standard <b>B (0.075 ng/ml)</b>	C1/2
100 µl	Standard <b>C (0.15 ng/ml)</b>	D1/2
100 µl	Standard <b>D (0.3 ng/ml)</b>	E1/2
100 µl	Standard <b>E (0.65 ng/ml)</b>	F1/2
100 µl	Standard <b>F (1 ng/ml)</b>	G1/2
100 µl	Control Serum <b>KS1</b>	H1/2
100 µl	Control Serum <b>KS2</b>	A3/4
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
<b>Incubation: 1 h at RT, 350 rpm</b>		
5x 300 µl	Aspirate the contents of the wells and wash 5x with <b>300 µl Wash Buffer WP</b>	each well
100 µl	Antibody-POD-Conjugate <b>AK</b>	each well
<b>Incubation: 1 h at RT, 350 rpm</b>		
5x 300 µl	Aspirate the contents of the wells and wash 5x with <b>300 µl Wash Buffer WP.</b>	each well
100 µl	Substrate Solution <b>S</b>	each well
<b>Incubation: 30 min in the Dark at RT</b>		
100 µl	Stopping Solution <b>SL</b>	each well
Measure the absorbance within <b>30 min</b> at <b>450 nm</b> with <b>≥590 nm</b> as reference wavelength.		

## Mediagnost Rat Adiponectin ELISA E091-R

Reagent preparation:	Reconstitution:	Dilution
<b>Standards A-F</b>	in 1 ml Dilution Buffer <b>VP</b>	
<b>Control Sera KS1 &amp; KS2</b>	in 250 µl Dilution Buffer <b>VP</b>	<b>1:1500 with Dilution Buffer VP</b>
<b>Washing Buffer WP</b>		<b>1:20 with Aqua. dest.</b> (e.g., add the complete contents of the flask ( <b>50 ml</b> ) into a graduated flask and fill with A.dest. to 1000 ml).
<b>Sample Dilution: e.g. 1 :1500</b>		
Pipette	Reagents	Position
100 µl	Dilution Buffer <b>VP</b>	A1/2
100 µl	Standard <b>A ( 0.25 ng/ml)</b>	B1/2
100 µl	Standard <b>B (0.75 ng/ml)</b>	C1/2
100 µl	Standard <b>C (1.5 ng/ml)</b>	D1/2
100 µl	Standard <b>D (3 ng/ml)</b>	E1/2
100 µl	Standard <b>E (6.5 ng/ml)</b>	F1/2
100 µl	Standard <b>F (10 ng/ml)</b>	G1/2
100 µl	Control Serum <b>KS1</b>	H1/2
100 µl	Control Serum <b>KS2</b>	A3/4
100 µl	Sample dilution	following wells
Cover the wells with the sealing tape.		
<b>Incubation: 1 h at RT, 350 rpm</b>		
3x 300 µl	Aspirate the contents of the wells and wash 3x with <b>300 µl Wash Buffer WP</b>	each well
100 µl	Antibody-POD-Conjugate <b>AK</b>	each well
<b>Incubation: 1 h at RT, 350 rpm</b>		
3x 300 µl	Aspirate the contents of the wells and wash 3x with <b>300 µl Wash Buffer WP</b>	each well
100 µl	Substrate Solution <b>S</b>	each well
<b>Incubation: 30 min in the Dark at RT</b>		
100 µl	Stopping Solution <b>SL</b>	each well
Measure the absorbance within <b>30 min</b> at <b>450 nm</b> with <b>≥590 nm</b> as reference wavelength.		