Version 07-11/24

User's Manual

Rat Adiponectin ELISA Kit

REF **DEIA-NB24-03R**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Rat Adiponectin ELISA Kit is intended to be used for quantitative measurement of Adiponectin in rat serum and plasma samples.

General Description

Adiponectin was described for the first time in the early 90th of the last century as an endocrine factor produced by adipocytes. Adiponectin is involved in regulation of energy- and fat metabolism. So, its concentration in the circulation is said to reflect the risk of atherosclerosis and the degree of insulin resistance. Based on the high incidence of these diseases, adiponectin was and still is object of intensive research regarding the underlying biological mechanisms and regarding its value as biomarker. Beside different cell culture models and studies with human samples, mice and rats are suitable model organisms for basic research and pre-clinical studies. Therefore, we developed and validated this test system as a tool for adiponectin measurements in rats usable in research and pre-clinical studies.

Principles of Testing

The Rat Adiponectin ELISA Kit is a so-called Sandwich-Assay using two specific and high affinity antibodies. The Adiponectin in the samples binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-Adiponectin-Antibody binds in turn to the immobilised Adiponectin. The second antibody is biotinylated and will be applied in a mixture with a Streptavidin-Peroxidase-Enzyme Conjugate. In the closing substrate reaction, the turn of the colour will be catalysed quantitatively depending on the Adiponectin-level of the samples.

Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with human anti-Rat Adiponectin antibody. Wells are separately breakable. (8x12) wells
2. **Standards (A-F):** lyophilized, (native Rat-Adiponectin), concentrations are given on vial labels and on the QC-certificate. 6 x 1 mL
3. **Control Serum 1 (KS1):** lyophilized, (rat serum), concentration is given on the QC-certificate. 1 x 250 µL
4. **Control Serum 2 (KS2):** lyophilized, (rat serum), concentration is given on the QC-certificate. 1 x 250 µL
5. **Dilution Buffer (VP):** ready for use. Please shake before use! 1 x 125 mL
6. **Antibody-POD-Conjugate (AK):** ready for use, contains a mixture of biotinylated anti-Adiponectin antibody and HRP (Horseradish Peroxidase)-labelled Streptavidin. 1 x 12 mL
7. **Washing Buffer (WP):** 20-fold concentrated solution, dilute 1:20 in A.dest. or in deionized Water. 1 x 50 mL
8. **Substrate (S):** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised Tetramethylbencidine. 1 x 12 mL
9. **Stopping Solution (SL):** ready for use, 0.2 M sulfuric acid. 1 x 12 mL
10. **Sealing Tape:** for covering the microtiter plate. 2

Materials Required But Not Supplied

1. Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer WP (A. dest.), 950 mL.
2. Precision pipettes and multichannel pipettes with disposable plastic tips
3. Polyethylene PE/Polypropylene PP tubes for dilution of samples
4. Vortex-mixer
5. Microtiter plate shaker (350 rpm)
6. Microplate reader ("ELISA-Reader") with filter for 450 and ≥590 nm

Storage

Store the kit at 2-8°C after receipt until its expiry date.

The shelf life of the components after initial opening is warranted for 4 weeks, store the unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The reconstituted components standards A-F and Control Sera KS1 and KS2 must be stored at –20°C (max. 4 weeks).

Attention: Standards should be thawed only once – where required please store aliquoted in adequate volumes. For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Avoid repeated thawing and freezing. The 1:20 diluted Washing Buffer WP is 4 weeks stable at 2-8°C.

Specimen Collection And Preparation

1. Sample type: Serum and Plasma

Influence of Heparin (30 IE/mL), EDTA (6.8 mM) and NaCitrat (0.015 M) on the measurement of Adiponectin has been investigated by recovery experiments. PBS was enriched with recombinant Rat Adiponectin and the above-mentioned substances. No significant influence on the recovery of adiponectin was detected.

2. Specimen collection

Hemolytic reactions have to be avoided. The blood has to be allowed to clot and after complete clotting, serum is separated by centrifugation.

3. Required sample volume: 10 µL recommended, minimum volume 5 µL

4. Sample stability

In firmly closable sample vials

• Storage at Room Temperature 20-25°C: max. 2 days

• Storage at -20°C: 2 years

• Freeze/Thaw cycles: max. 5

Freeze-Thaw cycles should be minimized. Up to 5 cycles showed no effect on the measured Adiponectin concentration.

Samples should be stored as fast as possible at least at + 4 ° C. For long-term storage, the sample must be frozen and stored at -20 ° C.

5. Sample dilution

• Dilution: 1:1500 with Dilution Buffer VP

We recommend a dilution in 2 steps:

Pipette 990 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 1:100) and mix each tube immediately. Pipette 100 µL of this dilution into another PE/PP vessel with 1400 µL of Dilution Buffer VP and mix immediately. This results in a final dilution of 1:1500. After mixing, use 100 µL per assay in the assay of this solution.

• If sample size is limiting, a minimum of 5 µL sample might be used alternatively, dilution in 495 µL Dilution Buffer VP yields a dilution of 1:100, Pipette 100 µL of this dilution into another PE/PP vessel with 1400 µL of Dilution Buffer VP and mix immediately. After Mixing use 100 µL per assay in the assay of this solution.

• Where required, depending on the expected Adiponectin values, the dilution with Dilution Buffer VP can be higher or lower.

Reagent Preparation

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming. Reagents with different lot numbers cannot be mixed.

1. The Standards A – F is reconstituted with 1mL Dilution Buffer VP. After resuspension, the standard is diluted according to a gradient - A (0.25 ng/mL), B (0.75 ng/mL), C (1.5 ng/mL), D (3.0 ng/mL), E (6.5 ng/mL) and F (10 ng/mL), which are prepared for immediate use.
2. The Control Sera KS1 and KS2 are reconstituted with 250 µL Dilution Buffer VP. After reconstitution dilute the Controls KS1 and KS2 with the Dilution Buffer VP in the same ratio (1:1500) as the sample.

Note: It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

3. The required volume of Washing Buffer WP is prepared by 1:20 dilution of the provided 20-fold concentrate with Aqua dest.

Assay Procedure

Note

When performing the assay, Standards A-F, Control Serum KS1&KS2 and the samples should be pipette as fast as possible (e.g.<15 minutes). To avoid distortions due to differences in incubation times, Antibody-POD-Conjugate AK as well as the succeeding Substrate Solution S should be added to the plate in the same order and in the same time interval as the samples. Stopping Solution SL should be added to the plate in the same order as Substrate Solution S. All determinations (Standards A-F, Control Sera KS1, KS2 and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

Incubation-Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate Solution S stabilized Tetramethylbencidine, is photosensitive–store and incubation in the dark.

Shaking-The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must be adjusted. Insufficient shaking may lead to inadequate mixing of

the solutions and thereby to low optical densities, high variations and/ or false values, excessive shaking may result in high optical densities and/ or false values.

Washing-Proper washing is of basic importance for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities e.g. high value of blank, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems. All washing must be performed with the provided Washing Buffer WP diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least. The danger of handling with potentially infectious material must be taken into account.

When using an **automatic microtiter** plate washer, the respective instructions for use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

Manual washing is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamical swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

Assay Step

1. Set Standard A-F, test samples (1:1500 diluted), Control Serum KS1 and KS2 (1:1500 diluted) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.
2. Prepare Standards: Aliquot 100ul of Standard A-F, Control Serum KS1/KS2 or test samples into wells.
3. Incubate: Seal the plate with a cover and incubate at 20-25°C, 350 rpm for 1 hour.
4. Wash: Aspirate the contents of the wells, and wash plate 3 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.
5. Add 100ul Antibody-POD- Conjugate AK into above wells (standard, control serum and test samples). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 20-25°C, 350 rpm for 30 minutes.
6. Wash: Aspirate the contents of the wells, and wash plate 3 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.
7. Add 100ul of Substrate Solution S into each well, cover the plate and incubate at 20-25°C in dark within 30 minutes.
8. Stop: Add 100ul Stopping Solution SL into each well.
9. Measure the absorbance within 30 min at 450 nm, with ≥ 590 nm as reference wavelength.

Quality Control

For the evaluation of the assay it is required that the absorbance values of the Blank should be below 0.25 and the absorbance of standard F should be above 1.00. Samples which yield higher absorbance values than

Standard F should be re-tested at a higher dilution.

Calculation

1. Calculate the mean absorbance value for the blank from the duplicated determination (well A1/A2).
2. Subtract the mean absorbance of the blank from the mean absorbances of all other samples and standards.
3. Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
4. Recommendation: Calculation of the standard curve should be done by using a computer program because the curve is in general (without respective transformation) not ideally described by linear regression. A higher-grade polynomial, or four parametric logistic (4-PL) curve fit or non-linear regression are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).
5. The Rat Adiponectin concentration in ng/mL of the samples and controls can be calculated by multiplication with the respective dilution factor.

Exemplary evaluation of sample concentrations

Sample dilution: 1:1500

Measured extinction of your sample.....0.6075

Measured extinction of the Blank.....0.0227

Your measurement program will calculate the Adiponectin concentration of the diluted sample automatically by using the difference of sample and Blank for the calculation. You only have to determine the most suitable curve fit. In this exemplary case the following equation is solved by the program to calculate the Adiponectin concentration in the sample:

$y = -0.0025x^2 + 0.2059x$

$R^2 = 0.9997$

x= 2.65

If the dilution factor (1500) is taken into account the Adiponectin concentration of the undiluted sample is:

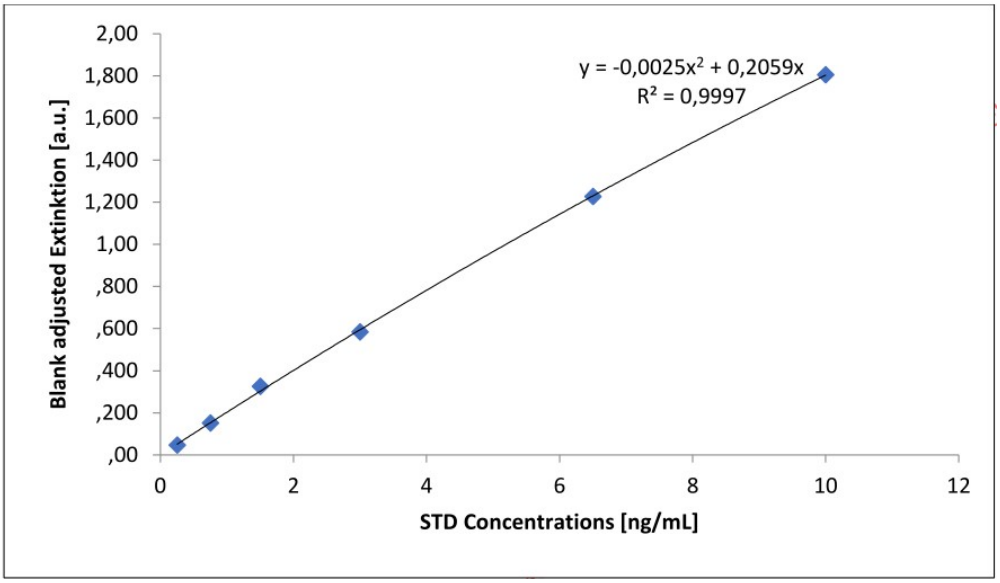
2.65 ng/mL x 1500 = 3975 ng/mL

= 3.975 µg/mL

Typical Standard Curve

The exemplary data and the standard curve in Figure 1 cannot be used for the calculation of the test results. You have to establish a standard curve for each test you conduct.

Standard	A	B	C	D	E	F
ng/mL	0. 25	0. 75	1.5	3	6.5	10
$OD_{450\text{-}620\ nm}$	0.047	0.152	0.326	0.585	1.227	1.805



Precision

The Inter- and Intra-Assay variation coefficients were on average ≤ 10%.

Intra-Assay-Variation

	Determinations [n]	Mean value [µg/mL]	SD	CV [%]
Sample 1	20	12.702	0.197	1.55
Sample 2	20	5.709	0.191	3.34

Inter-Assay Variation

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Mean	1.62	7.06	7.97	7.22	11.23	8.55	7.24
SD	0.03	0.40	0.27	0.28	1.25	0.42	0.35
CV [%]	2.16	5.70	3.39	3.82	11.16	4.93	4.85
n	6	6	6	6	6	6	6

Sensitivity

0.081 ng/mL

Precautions

1. For research and professional use only. The Creative Diagnostics kit is suitable only for in vitro use and not for internal use in humans and animals.
2. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Creative Diagnostics will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.
3. Do not use obviously damaged or microbial contaminated or spilled material.

4. This kit contains material of human and/or animal origin. Therefore all components and patient´s specimens should be treated as potentially infectious. (Rat Serum contained in the following components: KS1 and KS2, STD A-F)
5. Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.

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