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User's Manual

Mouse Adiponectin ELISA Kit

REF **DEIA-NB24-03M**

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 Mouse Adiponectin ELISA Kit is intended to be used for quantitative measurement of Adiponectin in mouse 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 patients, mice and rats are suitable model organisms for basic research and pre-clinical studies. Therefore we developed and validated this testsystem as a tool for adiponectin measurements in mice usable in research and pre-clinical studies.

Principles of Testing

The Mouse 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 anti-Mouse Adiponectin antibody. Wells are separately breakable. (8x12) wells
2. **Dilution Buffer (VP):** ready for use, please use for the reconstitution of Standards A-F, Control Sera KS1 & KS2 and for the serum dilution. 1 x 125 mL
3. **Standards (A-F):** lyophilized (native Mouse Adiponectin), Standard values are between 0.025 - 1 ng/ml (0.025, 0.075, 0.15, 0.3, 0.65 and 1 ng/ml). 6 x 1 mL

   Attention: If the standards are required for more than one assay process, we recommend to store the reconstituted Standards frozen at -20°C. Standards should be thawed only once – where required please store aliquoted in adequate volumes.
4. **Control Serum KS1&KS2:** lyophilized, (mouse serum). The dilution of the Control Sera KS 1&2 should be according to the dilution of the respective samples, the target value concentration should be obtained by multiplication with the respective dilution factor. 2 x 250 µL
5. **Antibody-HRP-Conjugate (AK):** ready for use, contains a mixture of biotinylated anti-Adiponectin antibody and HRP (Horseradish Peroxidase)-labelled Streptavidin. 1 x 12 mL

6. **Washing Buffer (WP):** 20-fold concentrated solution, dilute 1:20 in A.dest. or in deionized Water. 1 x 50 mL

   Attention: After dilution, the Washing Buffer is only 4 weeks stable, dilute only according to requirements.
7. **Substrate (S):** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised Tetramethylbencidine. 1 x 12 mL
8. **Stopping Solution (SL):** ready for use, 0.2 M sulfuric acid. 1 x 12 mL
9. **Sealing Tape:** for covering the microtiter plate. 2

Materials Required But Not Supplied

1. Precision pipettes and multichannel pipettes with disposable plastic tips
2. Distilled or deionized water for dilution of the Washing Buffer (WP)
3. Vortex-mixer
4. Microtiterplate shaker (350 rpm)
5. Microtiterplate washer (recommended)
6. Microplate reader ("ELISA-Reader") with filter for 450 and≥590 nm
7. Polyethylene PE/Polypropylene PP tubes for dilution of samples

Storage

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).

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.

Specimen Collection And Preparation

1. Serum and plasma samples of mice and mouse cell culture medium can be used in this assay. Influence of Heparin (30 IE/mL), EDTA (6,8 mM) and Sodium citrate (0,015 M) on the measurement of Adiponectin has been investigated be recovery experiments. PBS was enriched with recombinant mouse Adiponectin and the above-mentioned substances. No significant influence on the recovery of adiponectin was detected. Hemolytic reactions have to be avoided. The blood has to be allowed to clot and after complete clotting, serum is separated by centrifugation.
2. Storage: Store at RT for max. 2 days. Storage at -20°C for max. 2 years. Do not perfom more than five froze / thaw cycles.
3. Sample Preparation Samples have to be diluted in Dilution Buffer (VP). A sample dilution of 1:10 000 is in general suitable. However, the Adiponectin levels can vary individually significant, we would therefore recommend to check this and adjust the dilution respectively.

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 (0.025 ng/mL), B (0.075 ng/mL), C (0.15 ng/mL), D (0.3 ng/mL), E (0.65 ng/mL) and F (1 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 (1:10000) 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. The reconstituted standard and controls can be stored for 4 weeks at –20°C. Repeated freeze/thaw cycles have to be avoided.

3. The required volume of Washing Buffer WP is prepared by 1:20 dilution of the provided 20-fold concentrate with Aqua dest. The diluted Washing Buffer is stable for 4 weeks at 2-8°C. It has to be at room temperature for usage!

Assay Procedure

Note

1. All determinations (Standards, Control Sera KS1 & KS2 and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.
2. When performing the assay, the Standards, Control Sera and the samples should be pipetted as fast as possible (e.g.,<15 minutes).
3. All incubations have to conducted at room temperature (20-25°C).
4. To avoid distortions due to differences in incubation times, Antibody-POD-Conjugate AK as well as the following Substrate Solution S should be added to the plate in the same order and in the same time interval as the samples. Stop Solution SL should be added to the plate in the same order as the Substrate Solution.

Assay Step

1. Add 100 µl Dilution Buffer VP to wells A1 and A2 (Blank). Add 100 µl standard or 100 µl diluted control sera or diluted samples to the appropriate wells.
2. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at 350 rpm).
3. After incubation aspirate the contents of the wells and wash the wells 5 times 300 µl Washing Buffer WP / well. The washing buffer should incubate for at least for 15 seconds/cycle.
4. Following the last washing step pipette 100 µl of the Antibody-POD-Conjugate AK in each well.
5. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at 350 rpm).
6. After incubation wash the wells 5 times with Washing Buffer as described in step 3.
7. Pipette 100 µl of the TMB Substrate Solution in each well.
8. Incubate the plate for 30 minutes in the dark at room temperature (20 - 25°C).
9. Stop the reaction by adding 100 µl of Stopping Solution.
10. Measure the colour reaction within 30 minutes at 450 nm (reference filter ≥590 nm).

Calculation

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.25, these of standard F should be above 1.0.

Samples, which yield higher absorbance values than Standard F are beyond the standard curve, for reliable determinations these samples should be tested anew with a higher dilution.

Standards are provided in the following concentrations (use the concentration unit as preferred):

Standard	A	B	C	D	E	F
ng/mL	0.025	0.075	0.15	0.3	0.65	1

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 values.
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. 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 adiponectin concentration of the diluted controls KS1 & KS2 or the diluted samples is obtained from the standard curve. The multiplication of the respective calculated adiponectin content by the corresponding dilution factor then results in the adiponectin concentration of the undiluted starting solutions.

Precision

Intra-Assay Variability

	Determinations [n]	Mean value [µg/mL]	SD	CV [%]
Sample 1	21	7.395	159	2.15
Sample 2	21	1.712	37	2.16

Inter-Assay Variability

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Mean	1.12	6.12	7.65	8.04	2.15	7.54	6.87
SD	0.09	1.19	0.60	0.56	0.25	0.55	0.42
CV [%]	7.66	3.79	7.80	6.98	11.80	7.28	6.08
n	8	8	9	9	9	9	6

Sensitivity

0.008 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. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

3. Before use, all kit components should be brought to room temperature at 20 - 25°C. Precipitates in buffers should be dissolved before use by thorough mixing and warming.
4. Do not mix reagents of different lots. Do not use expired reagents.
5. This kit contains material of animal origin. Therefore all components and specimens should be treated as potentially infectious. 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. Do not use obviously damaged or microbial contaminated or spilled material. Mouse Serum contained in the following components: KS1 and KS2, STD A-F.