Version 08-11/24

User's Manual

Soluble Leptin Receptor (sLEP-R) ELISA Kit

REF **DEIA-NB24-20**

Σ **96T**

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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 sLEP-R ELISA Kit is intended to be used for quantitative measurement of soluble human leptin receptor (sLEP-R) in human serum or plasma for research purposes.

General Description

The adipokine leptin realizes signal transduction via four different leptin receptor (LEP-R) isoforms. The amount of functionally active LEP-R, however, is affected by constitutive shedding of the extracellular domain. The product of the cleavage process, the so-called soluble leptin receptor (sLEP-R, soluble Leptin receptor), is the main binding protein for leptin in human blood and modulates its bioavailability. Concentrations of sLEP-R are differentially regulated in metabolic disorders, such as type 1 diabetes mellitus or obesity and can therefore enhance or reduce leptin sensitivity. Lipotoxicity and apoptosis increase LEP-R cleavage via ADAM10-dependent mechanisms. In contrast, although increased sLEP-R concentrations seem directly to inhibit leptin effects, reduced amounts of sLEP-R may reflect decreased membrane expression of LEP-R. These findings, in part, explain alterations of leptin sensitivity that are associated with changes in serum sLEP-R concentrations seen in metabolic disorders.

Principles of Testing

The sLEP-R ELISA Kit is a so-called Sandwich-Assay using two specific and highly affine antibodies. The sLEP-R in the samples binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-sLEP-R antibody binds in turn to the immobilised sLEP-R. This is biotinylated and allows the binding of a streptavidin-peroxidase enzyme conjugate. Subsequently, the peroxidase catalyzes an enzymatic reaction resulting in a blue coloration. The intensity of the blue color depends on the sLEP-R content of the sample. The reaction is stopped by the addition of stop solution. After stopping the reaction, the color intensity (then yellow) is quantified by measuring the absorbance and converted to the sLEP-R concentration using a standard curve.

Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with human anti-hLeptin-R antibody. Wells are separately breakable. (8x12) wells
2. **Standards (A-H):** lyophilized, (recombinant hLeptin Receptor Standards), concentrations are given on vial labels and on quality certificate. 8 x 1 mL
3. **Control Serum 1 (KS1):** lyophilized, (human serum), concentration is given on quality certificate. 1 x 500 µL
4. **Control Serum 2 (KS2):** lyophilized, (human serum), concentration is given on quality certificate. 1 x 500 µL
5. **Antibody Conjugate (AK):** ready for use, contains biotinylated anti-hLeptin-R-antibody. 1 x 12 mL
6. **Enzyme Conjugate (EK):** contains Streptavidin-Peroxidase Conjugate. 1 x 12 mL

7. **Dilution Buffer (VP):** ready for use. Please shake before use! 1 x 60 mL
8. **Washing Buffer (WP):** 20-fold concentrated solution. 1 x 50 mL
9. **Substrate (S):** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised Tetramethylbencidine. 1 x 12 mL
10. **Stopping Solution (SL):** ready for use, 0.2 M sulfuric acid. 1 x 12 mL
11. **Sealing Tape:** for covering the microtiter plate. 3

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 lyophilized reagents should be stored at –20 °C after reconstitution. Avoid repeated thawing and freezing.

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-H and Control Sera KS1 and KS2 must be stored at –20°C (max. 4 weeks). For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Up to 3 of the freeze-thaw cycles did not influence the assay. 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

The measurement of corresponding Serum and Heparin or EDTA plasma samples gives comparable results, no influence of the anticoagulant was detectable.

2. Specimen collection

Use standard venipuncture for the blood sampling. Haemolytic reactions have to be avoided.

3. Required sample volume: 30 µL single determination / 50 µL double determination

4. Sample stability

In firmly closable sample vials

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

• Storage at 4°C: max. 3 days

• Storage at -20°C: 2 years

• Freeze/Thaw cycles: max. 3

Freeze-Thaw cycles should be minimized.

5. Interference

Neither triglycerides, bilirubin nor hemoglobin exert any influence on the measurement of sLEP-R in human serum up to concentrations of 100 mg/mL, 100 µg/mL and 1 mg/mL, respectively.

6. Sample dilution

• Dilution: e.g. 1:5 with Dilution Buffer VP

• Example for a double determination: Add 50 µL Sample to 200 µL Dilution Buffer VP (Dilution factor 5)

• Minimum required sample dilution 1:5

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 – H is reconstituted with 1 mL Dilution Buffer VP. After resuspension, the standards are diluted according to a gradient - A (0.0 ng/mL), B (0.625 ng/mL), C (1.25 ng/mL), D (2.5 ng/mL), E (5.0 ng/mL), F (10 ng/mL), G (20 ng/mL), H (30 ng/mL), which are prepared for immediate use.
2. The Control KS1 and KS2 are reconstituted with 500 µL Dilution Buffer VP. After reconstitution dilute the Control KS with the Dilution Buffer VP in the same ratio (1:5) 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

1. Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate S, stabilised Tetramethylbencidine, is photosensitive–store and incubation in the dark.
2. When performing the assay, Blank, Standards A-H, Control Serum KS1/2 and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Antibody Conjugate AK, Enzyme Conjugate EK as well as the succeeding Substrate 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 Substrate S. All determinations (Blank, Standards A-H, Control Serum KS1/2 and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.
3. Shaking: The incubation steps should be performed at mean rotation frequency of a microtiter plate shaker. We recommend 350 rpm. Depending on the design of the shaker, the shaking frequency should 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.

4. 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, 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.

   Automatic washing: When using an Automatic microtiter plate washer, the respective instructions fur 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: 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 Standards A-H, test samples (1:5 diluted), Control Serum KS1/2 (1:5 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. Aliquot 100ul of Standards A-H, Control Serum KS1/2 and test samples into wells.
3. Incubate: Seal the plate with a cover and incubate at 20-25°C, 350 rpm for 2 hours.
4. Wash: Aspirate the contents of the wells, and wash plate 5 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.
5. Add 100ul Antibody Conjugate AK into above wells. 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 2 hours.
6. Wash: Aspirate the contents of the wells, and wash plate 5 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.
7. Add 100ul Enzyme Conjugate EK into above wells. 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.
8. Wash: Aspirate the contents of the wells, and wash plate 5 times with 300 µL Washing Buffer WP. Do not let the wells dry completely at any time.
9. Add 100ul of Substrate Solution S into each well, cover the plate and incubate at 20-25°C in dark for 30 minutes.
10. Stop: Add 100ul Stopping Solution SL into each well.
11. Measure the absorbance within 30 min at 450 nm, with ≥ 590 nm as reference wavelength.

Quality Control

GLP requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. All kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated.

Quality criteria

For the evaluation of the assay it is required that the absorbance values of the Standard A should be below 0.25 and the absorbance of standard H should be above 1.00. Samples which yield higher absorbance values than Standard H should be re-tested at a higher dilution.

Calculation

1. Calculate the mean absorbance value for the Standard A from the duplicated determination (well A1/A2).
2. Subtract the mean absorbance of the Standard A 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 sLEP-R concentration in ng/mL of the samples and controls can be calculated by multiplication with the respective dilution factor.

Exemplary calculation of sample concentrations

Sample dilution: 1:5

Measured extinction of your sample 0.419

Measured extinction of Standard A 0.00

Your measurement program will calculate the sLEP-R concentration of the diluted sample automatically by using the difference of sample and Standard A 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 sLEP-R concentration in the sample:

$y = -3E\text{-}05x^3 + 0.0006x^2 + 0.078x$

$x = 5.17$

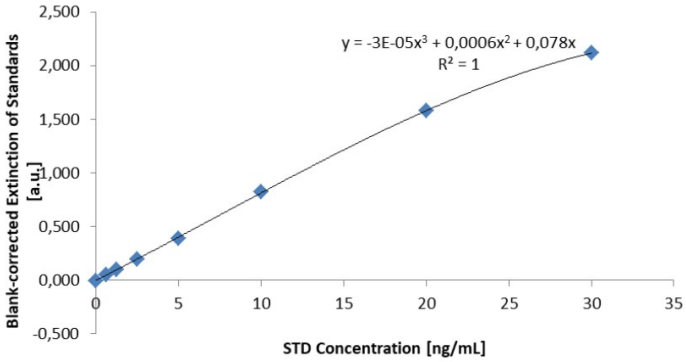
If the dilution factor (1:5) is taken into account the sLEP-R concentration of the undiluted sample is

$5.17 \times 5 = 25.85$ ng/mL

Typical Standard Curve

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

	A	B	C	D	E	F	G	H
ng/mL	0.0	0.625	1.25	2.5	5.0	10	20	30
$OD_{(450-620\ nm)}$	0.00	0.047	0.102	0.198	0.392	0.823	1.577	2.117



Precision

Intra-Assay Variance

Native human serum samples were measured (n=18) repeatedly at various positions of the microtiter plate. Intra-Assay variability was on average < 6%.

	Determinations [n]	Mean value [ng/mL]	SD	CV [%]
Sample 1	18	13.7	0.57	4.17
Sample 2	18	24.1	1.32	5.47
Sample 3	18	41.1	1.80	4.38

Inter-Assay-Variability

Serum samples were measured in independent tests. The coefficient of variation was 8% on average.

	Sample 1	Sample 2	Sample 3	Sample 4
Mean [ng/mL]	14.5	28.4	25.5	77.0
SD	0.96	3.01	1.26	5.18
CV [%]	6.63	10.57	4.95	6.73
n	17	17	16	16

Sensitivity

The analytical sensitivity of the test system is defined, as the concentration sLEP-R, that is distinguishable from the blank / background. Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the sLEP-R ELISA Kit is 0.0385 ng/mL on average. The limit of quantification (LOQ, as Serial dilutions with 3 different sera with max. difference of ± 20% to expected value) is 0.56 ng/mL.

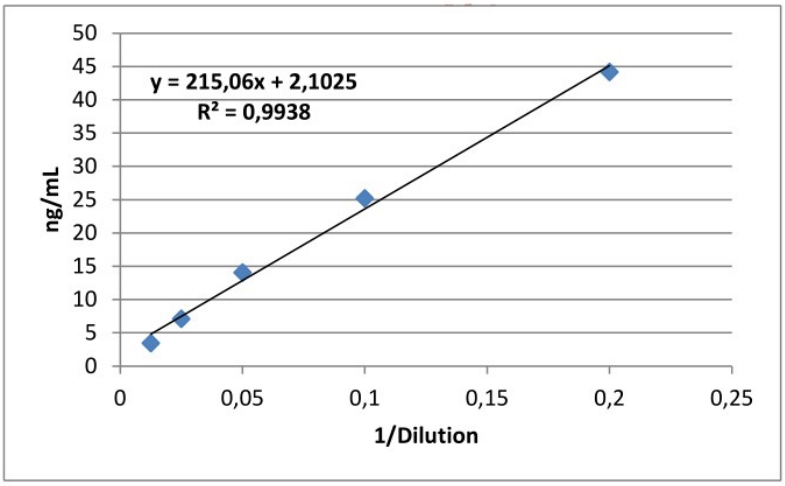
Specificity

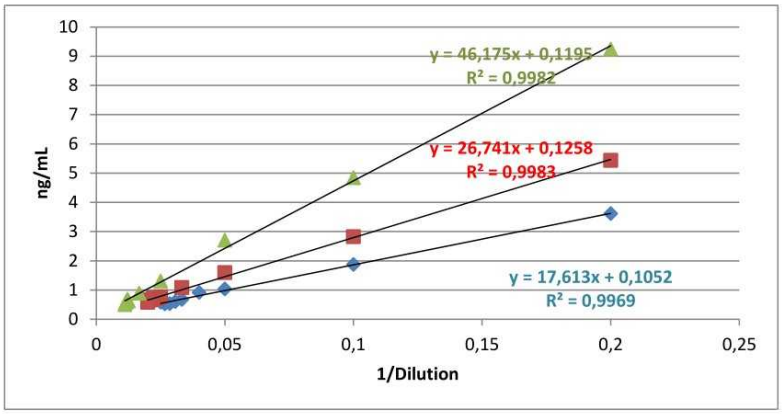
Rec. sLEP-R was added at concentrations of 10 ng/mL to the Dilution Buffer VP, to this rec. Leptin in

concentrations of 1 - 256 ng/mL was added. The sLEP-R content was measured: The measurement of rec. sLEP-R is not disturbed thorough rec. Leptin up to 256 ng/mL (values within +/-30%).

Linearity

The sLEP-R concentration of four human serum samples was determined at various dilutions. The results shown in Figure below prove that in the samples tested no influence of dilution can be detected. The linearity is given from the dilution 1:5 to 1:90.





Recovery

The recovery of recombinant sLEP-R in serum varied between 81 and 119% of the expected value.

Interferences

The possible interference of physiologically occurring substances was tested by adding different amounts of these potentially interfering substances to serum samples. Table below shows the relative recovery of sLEP-R in comparison to the serum without additions of these substances. None of the examined substances influenced the result of the test.

	Triglyceride (100 mg/mL)	Bilirubin (100 µg/mL)	Hemoglobin (1 mg/mL)
Sample 1	95	107	103
Sample 2	96	110	96
Sample 3	103	86	116

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.
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.
6. Following components contain human serum: Control Sera KS1, KS2

   Source human serum for the Controls provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore all components and patient´s specimens should be treated as potentially infectious.