Version 07-11/24

User's Manual

Mouse/Rat Growth Hormone ELISA Kit

REF **DEIA-NB24-26**

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Mouse/Rat Growth Hormone ELISA Kit is intended to be used for the measurement of Growth Hormone in mouse and rat serum and plasma samples for research use.

Principles of Testing

The Mouse/Rat Growth Hormone ELISA Kit is a so-called sandwich-assay. It utilizes two different specific high affinity polyclonal antibodies for this protein. The GH in the samples binds quantitatively to the immobilized antibody. In the following step, the biotinylated antibody in turn binds GH. After washing, a streptavidin-peroxidase-enzyme conjugate will be added, which will bind highly specific to the biotin of the antibody. Subsequently, the peroxidase catalyzes an enzymatic reaction resulting in a blue coloration. The intensity of the blue color depends on the GH content of the sample. The reaction is stopped by the addition of stop solution and color intensity is quantified by measuring the absorption.

Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with goat-anti-mouse/rat-GH-antibody. Wells are separately breakable. (8x12) wells
2. **Standards (A-G):** lyophilized, (recombinant rat GH), concentrations are given on vial labels and on quality certificate. 7 x 1 mL
3. **Control Serum 1 (KS1):** lyophilized, (rat serum), concentration is given on quality certificate. 1 x 150 µL
4. **Control Serum 2 (KS2):** lyophilized, (rat serum), concentration is given on quality certificate. 1 x 150 µL
5. **Antibody Conjugate (AK):** ready for use, guinea pig anti-m/r-GH-Antibody, biotinylated. 1 x 12 mL
6. **Enzyme Conjugate (EK):** ready for use, contains HRP (Horseradish-Peroxidase)-labeled Streptavidin. 1 x 12 mL
7. **Dilution Buffer (VP):** ready for use. Please shake before use! 1 x 50 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. Graduated cylinder for diluting Washing Buffer WP
3. Precision pipettes and multichannel pipettes with disposable plastic tips
4. Polyethylene PE/Polypropylene PP tubes for dilution of samples

5. Vortex-mixer
6. Microtiter plate shaker (350 rpm)
7. Microtiter plate washer (recommended)
8. 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-G 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 WB is 4 weeks stable at 2-8°C

Specimen Collection And Preparation

1. Sample type: Mouse and Rat Serum, Plasma.

EDTA-plasma samples of rats were found to be increased by plus 100% in a comparative study, relating to rat serum GH-values.

2. Specimen collection

Haemolytic reactions have to be avoided.

3. Required sample volume: 20 µL

4. Sample stability

• Sample transport is recommended chilled e.g. on cooling elements (blue ice) or frozen on dry ice.

• in firmly closable sample vials

• Storage at -20°C: min. 2 years

• Freeze/-thaw cycles: max. 10

5. Sample dilution

For commercial pooled rodent sera a 1:5 dilution was found suitable.

An extraction step is not required.

• Dilution: with Dilution Buffer VP:

For a double determination: e.g. 50 µL sample plus 200 µL Dilution Buffer VP

• After mixing use 100 µL diluted sample per well in the assay within 1 hour of this solution.

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

Depending upon the used strain of the animals or the experimental conditions, the endogenous content of GH can vary strongly. It is recommended to test in advance the individual optimal sample pre-dilution under

the respective conditions.

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 – G is reconstituted with 1 mL Dilution Buffer VP. After resuspension, the standards are diluted according to a gradient - A (0.15 ng/mL), B (0.45 ng/mL), C (0.90 ng/mL), D (1.8 ng/mL), E (3.6 ng/mL), F (6.0 ng/mL), G (9.0 ng/mL), which are prepared for immediate use.
2. The Control Sera KS1 and KS2 are reconstituted with 150 µL Dilution Buffer VP. After reconstitution dilute the Control Sera KS1 and KS2 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. Attention: Standards should be thawed only once – where required please store aliquoted in adequate volumes.

3. The required volume of Washing Buffer WP is prepared by 1:20 dilution of the provided 20-fold concentrate with Aqua dest. The wash buffer WP, diluted 1:20, is stable for 4 weeks.

Assay Procedure

Note

1. Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate S, stabilised H_2O_2 Tetramethylbencidine, is photosensitive–store and incubation in the dark.
2. When performing the assay, Blank, Standards A-G, Control Sera KS1 and 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 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-G, Control Sera KS1 and KS2 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. Substrate S Incubation without shaking.
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-G, test samples (1:5 diluted), Control Serum KS1 and KS2 (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 Dilution Buffer VP (Blank), Standards A-G, Control Serum KS1 and KS2 and 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 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 1 hour.
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 15 min at 450 nm, with ≥ 590 nm as reference wavelength.

Quality Control

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.30, these of standard G should be above 1.0.

Samples, which yield higher absorbance values than Standard G are beyond the standard curve, for reliable determinations these samples should be tested anew with 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 absorbance 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 m/rGH concentration of the diluted sample or the diluted control sera KS1&2 in ng/mL is calculated in this way, the m/rGH concentration of the undiluted sample and of KS1 & KS2 is calculated by multiplication with the respective dilution factor.

Exemplary calculation of GH concentrations

Sample dilution: 1:5

Measured extinction of your sample 1.5

Measured extinction of the blank 0.1356

Your measurement programm will calculate the m/rGH 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 (here: polynomial 2nd degree).

In this exemplary case the following equation is solved by the program to calculate the GH concentration in the sample:

$y = -0.0041x^2 + 0.2896x$

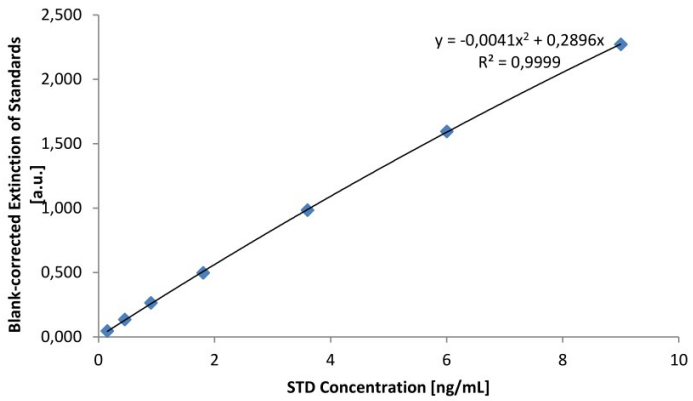
$x = 5.09$

Multiplication by dilution factor (5) gives the GH concentration of the sample with 25.45 ng/mL.

Typical Standard Curve

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

	Blank	A	B	C	D	E	F	G
ng/mL	0	0.15	0.45	0.90	1.8	3.6	6.0	9.0
$OD_{(450\text{-}620\ nm)}$	0.1356	0.182	0.272	0.4	0.634	1.121	1.732	2.408



Precision

The Inter- and Intra-Assay variation coefficients were on average <10% and <5%.

Inter-Assay-Variation (n=7)

	Mean value [ng/mL]	SD	CV [%]
Sample 1	9.84	0.73	7.41
Sample 2	15.77	0.86	5.48

Intra-Assay-Variation (n=12)

	Mean value [ng/mL]	SD	CV [%]
Sample 1	10.03	0.32	3.22
Sample 2	3.74	0.17	4.55
Sample 3	16.16	0.33	2.01

Sensitivity

The analytical sensitivity of the Mouse/Rat Growth Hormone ELISA Kit was measured by the variability of the signal of the blank (by 15 to 16-fold determinations). Based on the twofold standard deviation of the blank the mean analytical sensitivity is < 0.04 ng/mL (Range 0.014 to 0.054 ng/mL).

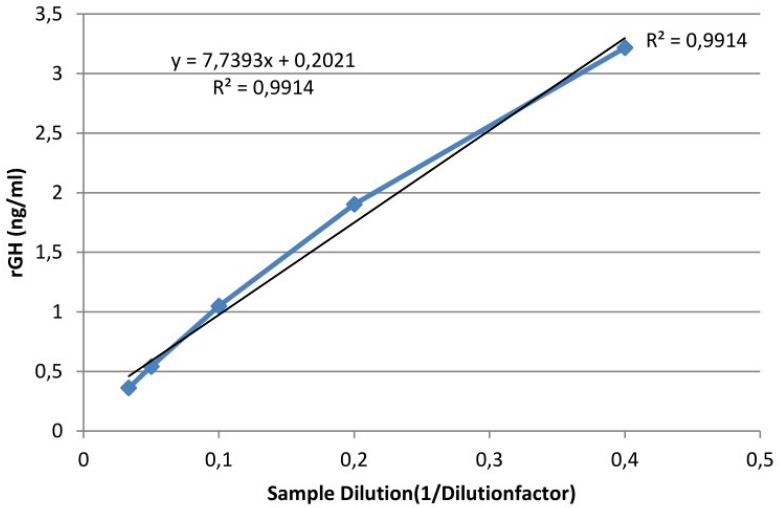
Specificity

Serum of the different species were used as diluted samples in this assay system. No cross reactivity was detected for: Rabbit, Guinea pig, Dog, Cat, Chicken, Sheep, Goat, Pig, Donkey, Horse and Bovine.

No Cross reactivity was measured with recombinant human eukaryotic expressed GH (at 1 µg/mL)

Linearity

Linearity of sample dilution was tested by serial dilution of 3 rat sera. No diluted sample showed a relative standard deviation of >15 % in comparison to the respective mean rGH concentration. Linearity of sample dilution is shown by linear regression in the dilution range of 1:2.5 - 1:30. We recommend preferentially a dilution of 1:5. Alternatively e.g. dilutions from 1:2.5 up to 1:30 (in case of higher rGH levels) dilutions would be suitable.



Rat serum samples were diluted in VP and rGH content was calculated. Measurements results are shown in [ng/mL]. No Coefficient of Variation >15 % was detected.

Dilution	Sample 1 [ng/ml]	Sample 2 [ng/ml]	Sample 3 [ng/ml]
1:2.5	15.1	8.0	3.968
1:5	17.7	9.5	4.62
1:10	19.6	10.5	5.329
1:20	20.8	10.8	5.508
1:30	21.3	10.8	-
AV / SD / CV%	**18.9 / 2.5 / 13.2**	**9.93 / 1.189 / 11.95**	**4.9 / 0.7 / 14.4**

Interferences

Interference of physiological appearing Hemoglobin with the m/rGH measurement was investigated. Serum samples have been enriched with different concentrations of possibly interfering Hemoglobin and the amount of m/rGH was measured and compared with the m/rGH concentration in the same sample without any enrichment. Hemoglobin did not interfere significantly with m/rGH measurement.

	Hemoglobin (5 mg/mL)
Sample 1	89
Sample 2	94
Sample 3	114

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 animal serum: KS1, KS2