Creative Diagnostics®

Version 07-11/24

User's Manual

Human Growth Hormone ELISA Kit

REF **DEIA-NB24-25**

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 Human Growth Hormone ELISA Kit is intended to be used for quantitative measurement of human growth hormone ELISA Kit in human serum and plasma for research purposes.

In addition to serum and plasma samples, hGH can be determined in other human body fluids and in cell culture supernatants of various human cell lines for research purposes

General Description

The endocrine system of human Growth Hormone (hGH), also named Somatropin, is characterized by an extreme complexity. hGH is the product of the GH-1 gene located on chromosome 17 and expressed in pituitary cells. 80% of the hGH is a non-glycolsylated 22 kDa protein consisting of 191 amino acids. About 20% is a variant form of 20 kDa resulting from alternative splicing. Additionally, several smaller variants can be found in circulation as well as translational modificated proteins and different degrees of protein aggregation. Bioactivity of Growth Hormone is regulated by a specific binding protein (GHBP) formed by the extra cellular part of the cellular transmembran GH-receptor. These modifications allow a tight control of the half-life period hGH and of its bioactivity.

Not only synthesis and posttranslational modification but also secretion of hGH is tightly regulated. Spontaneous pulsatile secretion takes place with a single pulse every three hours and a maximal secretion during night's sleep. Several different stimuli as physiologic stress or hypoglycaemia result in additional hGH secretion, induced by the hypothalamic hormones Somatostatin and GH-Releasing Hormone (GHRH). Age, sexual steroids, nutritional status, illness and emotions influence the amount of secreted hGH. Because of the multitude of influencing factors, the normal quantitative secretion is not known.

Physiological functions are partially exerted by Insulin-like Growth Factors (IGFs). In children and adolescent, the hGH system is the main regulator of growth. If the hGH system fails totally, human growth will end at 120 cm. Beside regulation of growth hGH exerts an anabolic effect on muscle and connective tissue as wells as on bone and different other organs (heart, intestine). Further hGH was proved to have a lipolytic effect.

Growth Hormone pathology is characterized by extreme high or extreme low hGH secretion. During childhood it is the Growth Hormone deficiency congenital or acquired, which leads to microsomia. For diagnosis of Growth Hormone deficiency an hGH stimulation test has to be done or the spontaneous excretion must be investigated. The therapy consists of substitution of endogenous Growth Hormone by recombinant hGH resulting in normalization of growth.

In adulthood hGH deficiency is mostly caused by pituitary adenoma (and their surgical excision). hGH deficiency is associated with adipositas, muscle dystrophy, arteriosclerosis, osteoporosis, adynamia. Substitutional therapy is a well-known, approved and efficient therapy of severe Growth Hormone deficiency in adulthood. Therapeutic success is directly as well as indirectly proved by measurement of IGF in serum.

Excessive hGH secretion, mostly caused by pituitary adenoma, results in childhood in gigantism, in adulthood in acromegaly, leading to enlarged extremities, diabetes, heart insufficiency and tumor growth. Surgical excision of the adenoma is the therapy of choice. If tumor excision is not possible or incomplete, a medicinal therapy with somatostatin preparation will be conducted, resulting in inhibition of hGH production. Alternatively, hGH analoga (e.g. Pegvisomat) are used to block the hGH receptor and thereby inhibit action of endogenous hGH. Measurement of human Growth Hormone (hGH, Somatropin) is done for diagnostic of

Growth Hormone deficiency or Growth Hormone excess (arcomegaly). During medicinal and/or after surgical therapy of arcomegaly Growth Hormone (and IGF-I) measurement is used for therapy control.

Principles of Testing

The Human Growth Hormone ELISA Kit is a so-called sandwich-assay. It utilizes a specific, high affinity polyclonal rabbit antiserum coated on the wells of a microtiter plate. The hGH in the samples binds quantitatively to the immobilized antiserum. In the following step, the biotinylated antibody in turn binds hGH. After washing, a streptavidin-peroxidase-enzyme conjugate will be added, which will bind highly specific to the biotin of the antibody and will catalyze the substrate to change the color quantitatively depending on the hGH level of the sample.

Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with rabbit-anti-hGH-antibody. Wells are separately breakable. (8x12) wells
2. **Calibrators (CAL A-E):** lyophilized, (recombinant human GH), concentrations are given on vial labels and on quality certificate. 5 x 750 µL
3. **Control (CTR):** lyophilized, (human serum), concentration is given on quality certificate. 1 x 500 µL
4. **Antibody Conjugate (DET):** ready for use, contains rabbit biotinylated anti-hGH antibody. 1 x 12 mL
5. **Enzyme Conjugate (EC):** ready for use, contains HRP (Horseradish-Peroxidase)-labelled Streptavidin. 1 x 12 mL
6. **Dilution Buffer (DIL):** ready for use. Please shake before use! 1 x 120 mL
7. **Washing Buffer (WB):** 20-fold concentrated solution. 1 x 50 mL
8. **Substrate (S):** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised Tetramethylbencidine. 1 x 12 mL
9. **Stopping Solution (STP):** 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. Microtiter plate washer (recommended)
7. 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 Calibrators A-E and Controls CTR 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: Serum and Plasma

Serum and Heparin/EDTA Plasma yield comparable values. The hGH levels are reduced in citrate plasma samples, because of the relatively high amount of anticoagulant.

2. Specimen collection

Human GH is secreted pulsatile during the day/night. Therefore, in clinical application stimulation test are used to measure peak GH concentrations.

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

3. Required sample volume: 10 µL

4. Sample stability

In firmly closable sample vials

• Storage at 20-25°C: 3 days

• Storage at -20° C: min. 2 years

• Freeze-thaw cycles max. 5

The storage of samples over a period of 2 years at -20°C, showed no influence on the reading. Freezing and thawing of samples should be minimized.

5. Interference

Triglyceride and bilirubin in the sample do not interfere to a concentration of 100 mg/mL and 200 µg/mL. However, the use of hemolytic, lipemic or icteric samples should be validated by the user.

6. Sample dilution

• Dilution: 1:26 with Dilution Buffer DIL

• Pipette 250 µL Dilution Buffer DIL in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series); add 10 µL sample (dilution 1:26). After mixing use 2 x 100 µL of this dilution in the assay.

• Sample stability after dilution of the sample: at least 1 hour at 20-25°C.

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 Calibrators A – E is reconstituted with 750 µL Dilution Buffer DIL. After resuspension, the calibrators

are diluted according to a gradient - A (0.05 ng/mL), B (0.15 ng/mL), C (0.3 ng/mL), D (0.6 ng/mL), E (1.0 ng/mL), which are prepared for immediate use.

2. The Controls CTR are reconstituted with 500 µL Dilution Buffer DIL. After reconstitution, dilute the Controls CTR with the Dilution Buffer DIL in the same ratio (1:26) 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 WB 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, Calibrators A-E, Controls CTR 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 DET and the Enzyme Conjugate EC 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 STP should be added to the plate in the same order as Substrate S. All determinations (Blank, Calibrators A-E, Controls CTR 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. 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 WB 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. Pipette in positions A1/2 100 µl Dilution Buffer DIL (Blank).
2. Pipette in positions B1/B2 100 µl Calibrator A (0.05 ng/mL).

   Pipette in positions C1/C2 100 µl Calibrator B (0.15 ng/mL).

   Pipette in positions D1/D2 100 µl Calibrator C (0.3 ng/mL).

   Pipette in positions E1/E2 100 µl Calibrator D (0.6 ng/mL).

   Pipette in positions F1/F2 100 µl Calibrator E (1.0 ng/mL).
3. Pipette in positions G1/G2 100 µl of the Control CTR (1:26 diluted).
4. Add 100 µl Sample (1:26 diluted) in the rest of the wells according to requirements.
5. Cover the wells with sealing tape and incubate the plate for 2 hours at 20-25°C, 350 rpm.
6. After incubation aspirate the contents of the wells and wash the wells 5 times 300 µl Washing Buffer WB / well.
7. Following the last washing step pipette 100 µl of the Antibody Conjugate in each well.
8. Cover the wells with sealing tape and incubate the plate for 30 minutes at 20-25°C, 350 rpm.
9. After incubation, pipette 100 µl of the Enzyme Conjugate in the wells the previously pipetted Antibody Conjugate DET, thereto mix shortly through cautious tapping on the side of the MTP. Attention: high filled volume of the wells!
10. Cover the wells with sealing tape and incubate the plate for 30 minutes at 20-25°C, without shaking.
11. After incubation wash the wells 5 times with Washing Buffer WB as described in step 6.
12. Pipette 100 µl of the Substrate Solution S in each well.
13. Incubate the microtiter plate for 15 minutes in the dark at room temperature (20°C -25°C).
14. Stop the reaction by adding 100 µl Stopping Solution STP to all wells.
15. Measure the absorbance within 30 minutes at 450 nm (Reference filter ≥ 590 nm).

Quality Control

Good laboratory practice requires that controls are included in each assay. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The test results are only valid if the test has been performed following the instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state or local standards/laws. All calibrators and 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. Each laboratory should use known samples as further controls.

Quality criteria

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 Calibrator E should be above 1.00. Samples, which yield higher absorbance values than Calibrator E, should be re-tested with a higher dilution.

Calculation

The 2nd International Standard for hGH, NIBSC Code 98/574 (6), was used as calibration material. This was defined in an international study in the year 2001 with 3 International units per mg Protein (3 IU/mg). The exclusive application of this calibration material is recommended in line with the current standardisation efforts for hGH Immunoassays.

Calibrator	A	B	C	D	E
ng/mL	0.05	0.15	0.30	0.60	1.0
pg/mL	50	150	300	600	1000
µIU/mL	0.15	0.45	0.9	1.8	3.0

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, calibrators and control.
3. Plot the calibrator concentrations on the x-axis versus the mean value of the absorbance of the calibrators on the y-axis.
4. Recommendation: Calculation of the calibration 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 hGH concentration in ng/mL (or pg/mL, or µIU/mL, according the chosen unit for the calibrators) of the samples can be calculated by multiplication with the respective dilution factor.

Exemplary calculation of GH concentrations

Sample dilution: 1:26

Measured extinction of your sample 0.25

Measured extinction of the blank 0.08

Your measurement programm will calculate the hGH 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 3rd degree).

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

$0.17 = -1.2868x^3 + 0.8619x^2 + 3.0523x + 0.0532$

$x = 0.035$

If the dilution factor (1:26) is taken into account, the hGH concentration of the undiluted sample is

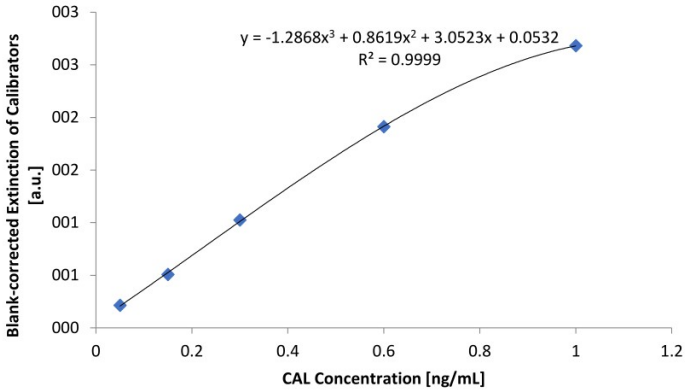
$0.035 \times 26 = 0.91$ ng/mL

Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generation at the time of assay.

	Blank	A	B	C	D	E
ng/mL	0	0.05	0.15	0.3	0.6	1.0
$OD_{(450-620\ nm)}$	0.08	0. 2955	0.5885	1.1045	1.9935	2.761

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



Precision

Intra-Assay Variance

One sample has been measured 14 times in the same assay. The results are shown in table below. The measured coefficient of variation (CV) is 5.46%. Intra assay variance has also been evaluated externally, two serum samples with 0.45 and 5.94 µg/L hGH were measured 10 times within the same assay. The resulting coefficients of variation were 3.65% and 2.16%.

	Determinations [n]	Mean value [µg/L]	SD	CV [%]
Sample 1	16	2.41	0.19	7.99
Sample 2	16	5.84	0.27	4.70
Sample 3	16	14.98	0.55	3.70

Inter-Assay Variance

Serum samples were measured in independent assays. On average the coefficient of variation was 4.34%. Results are shown in detail in table below. Here also externally acquired data are available: The mean coefficient of variation for inter-assay variance at 2.39; 5.37 and 14.33 µg/L hGH was 5.98%; 3.93% and 3.12%, respectively.

	Determinations [n]	Mean value [µg/L]	SD	CV [%]
Sample 1	14	5.37	0.21	3.93
Sample 2	10	2.39	0.14	5.98
Sample 3	11	14.33	0.45	3.12

Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the Human Growth Hormone ELISA Kit is 0.0115 µg/L.

Specificity

Cross reactivity with recombinant human Prolactin has been tested and no significant signal was measured in an enriched serum sample containing 200 µg/L Prolactin.

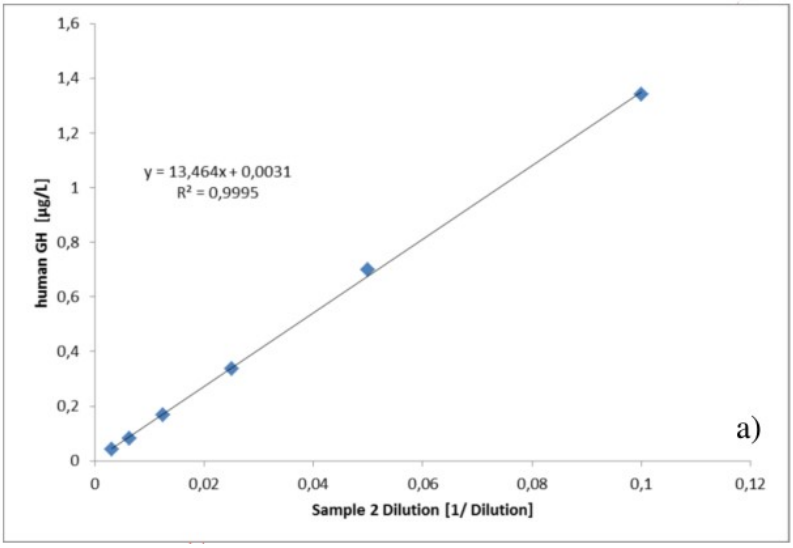
Further, Pegvisomant (trade name Somavert), a growth hormone analogue and drug used in acromegaly therapy, has been tested for cross-reactivity in assay buffer in different concentrations. Here no significant influence of Pegvisomant was detected.

Several commercially available animal sera have been tested as samples in different dilutions (1:5 or 1:26) in this assay. No signal was detected in serum of the following species: donkey, dog, goat, guinea pig, horse, rat, mouse, rabbit, sheep, cat, chicken and horse.

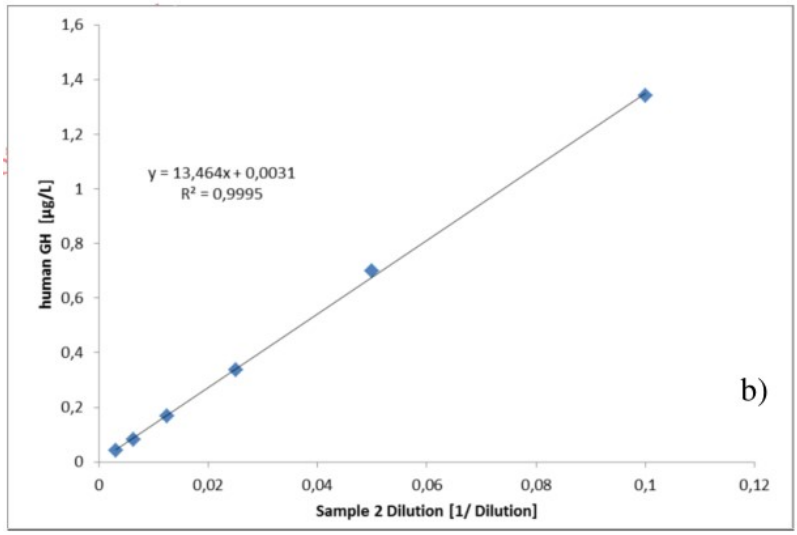
Whether this obvious non-reactivity is species specific should be assessed individually by each customer. We remind each customer that hGH secretion is pulsatile and thus commercially available animal serum samples may not be taken at the ideal daytime. An external laboratory was able to find good measurable signals in bovine serum.

Linearity

Linearity of the Human Growth Hormone ELISA Kit was tested by dilution of 2 different serum samples. The samples were diluted in the range of 1:10 to 1:76800. Linearity of sample dilution was shown by linear regression in the dilution range of 1:10 - 1:9600.



a) 1:600 - 1:9600



B) 1:10 - 1:32

Recovery

Recombinant human Growth Hormone (NIBSC 98/574) was added in different amounts to human serum. The hGH content of the so enriched samples was measured and recovery in comparison to enriched Dilution Buffer (DIL) calculated.

NIBSC Rec. hGH	DIL		Serum 1		Serum 2	
ng/mL	ng/mL	%	ng/mL	%	ng/mL	%
20	19.34	96.7	17.51	83.2	17.53	81.9
10	9.81	98.1	9.28	84.0	9.18	80.4
5	5.34	106.7	4.99	82.67	5.9	92.0
0	0	-	1.04	-	1.41	-

Interferences

Interference of bilirubin and triglycerides has been tested. Here neither bilirubin (up to 200 mg/L) nor triglycerides (up to 100 g/L) showed a significant interference with hGH measurement.

Bilirubin [mg/L]	hGH Recovery [%]	Triglycerides [g/L]	hGH Recovery [%]
25	111	12.5	89
50	116	25	109
100	112	50	85
200	108	100	110

We also tested the influence of growth hormone binding protein up to 10 µg/L on hGH measurement and haven´t seen a significant effect (mean recovery 98%).

	hGH [µg/L]		
	2	**8**	**20**
GHBP [µg/L]	1	1	1
hGH Recovery [%]	95	95	99
GHBP [µg/L]	5	5	5
hGH Recovery [%]	87	95	97
GHBP [µg/L]	10	10	10
hGH Recovery [%]	87	93	95

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 CTR

   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.

Limitations

The Human Growth Hormone ELISA Kit is based on polyclonal rabbit antibodies. Generally, this technique is sensible to heterophilic antibodies in the sample. The influence of heterophilic antibodies is reduced by assay design, but cannot be excluded completely.

Interference of several physiological and pharmaceutical substances has been tested for the indicated concentrations. Higher concentrations or other substances may interfere with the measurement.