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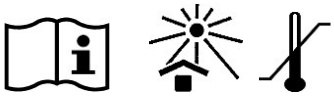
Instructions for Use

Growth hormone ELISA

Enzyme immunoassay for the direct quantitative determination of human Growth hormone in human serum.

REF **DB59121**

Σ **96**



EU: IVD CE U.S.: *For research use only.*
Not for use in diagnostic procedures.

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1. INTENDED USE

Enzyme immunoassay for the direct quantitative determination of human Growth hormone in human serum.

2. SUMMARY AND EXPLANATION

Biological activities

hGH is a polypeptide hormone (molecular weight 21 500 Da) produced by the acidophil cells of the anterior pituitary under the control of two main substances from the median eminence : Growth-hormone Releasing Factor (GRF) and an inhibitory agent, somatostatin. Dopaminergic, adrenergic and serotoninergic neuroendocrine pathways also play an important role in the control of hGH secretion. Excitatory stimuli of hGH secretion include hypoglycemia, exercise, fasting, meals with a high protein content, deep sleep, stress, glucagon, L Dopa, amino acids, etc. Inhibitory stimuli include glucose, cortisol, hGH and free fatty acids. Because of its short plasma half life (±25 minutes) and of the frequent excitatory or inhibitory stimuli, hGH displays frequent and large variations of concentration in serum.

One of the main physiological functions of hGH is to act on the liver and other tissues to produce somatomedins, which in turn induce growth by direct action on target tissues. In contrast to hGH, the concentration of somatomedins in serum is kept stable by virtue of being largely bound to circulating plasma proteins.

Clinical application

Growth retardation: hGH hyposecretion is one of the various causes of small stature in children. Serum hGH measurement with a highly sensitive assay, especially following a provocative stimulus (absence of response), is an important way to establish this diagnosis because this group of patients can be treated by administration of hGH.

Hypopituitarism: Serum hGH measurement is also an index of pituitary function when hypopituitarism (either idiopathic or due to tumour and surgery) is suspected.

Gigantism and acromegaly: Serum hGH measurement, especially following a provocative inhibitory test (absence of response), is an important way to establish the diagnosis of hGH hypersecretion due to acidophilic pituitary tumour. This results in gigantism in children and acromegaly in adults. Both of these disorders may be treated by surgery or radiation.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with an antibody, directed towards an epitope of an antigen molecule. The antigen of the sample is incubated in the coated well with enzyme conjugated second antibody (E-Ab), directed towards a different region of the antigen molecule. After the substrate reaction the intensity of the developed color is proportional to the amount of the antigen. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
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. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.

10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8 °C.

Once opened standards and controls should be used within 14 days or aliquoted and stored frozen.

6. SPECIMEN COLLECTION AND STORAGE

Serum

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Do not use specimens containing NaN_3 or Thimerosal, as they may lead to false results. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage	4°C	≤ -10°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability	24 h	≥ 24 h	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with antibodies against hGH (monoclonal).
1 x 0.2 mL	ENZCONJ CONC	**Enzyme Conjugate,** Concentrate (100 x) Contains: hGH-HRP Conjugate, in protein-containing buffer, non-mercury preservatives.
1 x 2 mL	CAL A	**Standard A-F** 0; 1; 5; 10; 25; 50 ng/mL Ready to use. Contains: hGH in serum-containing buffer, non-mercury preservatives. Exact concentrations see vial labels or QC certficate. The Standards of this assay have been adjusted to WHO-Standard. (WHO) 1st IS 80/505.
1 x 5 x 0.5 mL	CAL B-F	
1 x 0.5 mL	CONTROL LOW	**Positive Control** Ready to use. Contains: hGH in serum-containing buffer, non-mercury preservatives. Exact concentrations see vial labels or QC certficate.
1 x 0.5 mL	CONTROL HIGH	**Negative Control** Ready to use. Contains: hGH in serum-containing buffer, non-mercury preservatives. Exact concentrations see vial labels or QC certficate.
1 x 16 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB and hydrogen peroxide in a non-DMF or DMSO containing buffer.
1 x 6 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4.
1 x 50 mL	WASHBUF CONC	**Wash Buffer,** Concentrate (10 x) Contains: Buffer with non-ionic detergent and non-mercury preservatives.
1 x 15 mL	ASSAYBUF	**Assay Buffer** Ready to use. Contains: protein-containing buffer, non-mercury preservatives.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 25; 50; 100; 300 µL
2. Vortex mixer
3. Orbital shaker (600 rpm) (e.g. EAS 2/4, SLT) or Linear shaker (200rpm)
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Some components contain ≤ 250 µL solution. Take care that the solution is completely on the bottom of the vial before opening.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
6. Use a pipetting scheme to verify an appropriate plate layout.
7. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
8. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
9. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of concentrated components

The contents of the kit for 96 determinations can be divided into 3 separate runs.
The volumes stated below are for one run with 4 strips (32 determinations).

Dilute / dissolve	Component	with	Diluent	Relation	Storage	Stability
15 mL	WASHBUF CONC	135 mL	bidist. water	1:10	2-8°C	12 months
40 µL	ENZCONJ CONC	4 mL	ASSAYBUF	1:100	Discard any that is left over.	

11. TEST PROCEDURE

1.	Pipette **25 µL** of each **Calibrator, Control and sample** into the respective wells of the Microtiter Plate.
2.	Pipette **100 µL** of freshly prepared **Enzyme Conjugate (1:100)** into each well.
3.	**Incubate 1 h** at **RT (18-25°C)** on an orbital shaker (approx. 600 rpm) or linear shaker (200 rpm).
4.	Wash plate **3 x** with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
6.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
7.	**Incubate 10 - 15 min** at **RT (18-25°C)** on an orbital shaker (approx. 600 rpm) or linear shaker (200 rpm) or until calibrator F attains dark blue colour for desired OD.
8.	Stop the substrate reaction by adding **50 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
9.	**Measure** optical density with a photometer at **450 nm*** within **20 min**.

*) If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

12. QUALITY CONTROL

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 comparable standards /laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.
In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistics or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

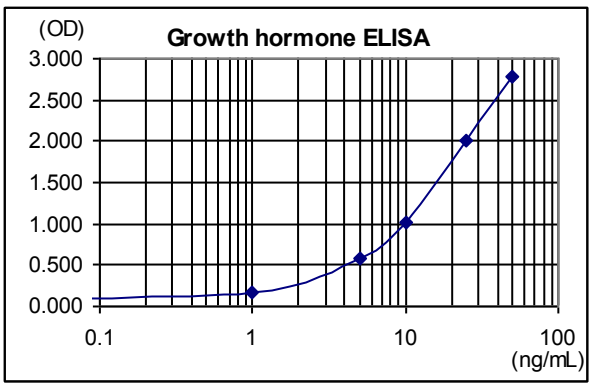
The concentration of the samples can be read directly from the standard curve.

Samples containing concentrations higher than the highest standard have to be diluted up to 1:10 with Standard A and reassayed. The result obtained should be multiplied by the dilution factor.

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	OD_1	OD_2	OD_{Mean}	Value (ng/mL)
A	0.074	0.072	0.073	0
B	0.158	0.159	0.159	1
C	0.574	0.580	0.577	5
D	0.997	1.014	1.006	10
E	2.021	2.009	2.015	25
F	2.809	2.773	2.791	50



14. EXPECTED VALUES

It is recommended that each laboratory establishes its own range of normal values.

Group		N	Mean (ng/mL)	Range (ng/mL)
Females	Premenopausal	17	2.2	0.0 – 12.5
	Postmenopausal	9	2.6	0.0 – 13.5
Males		16	1.9	0.0 – 6.8

15. PERFORMANCE

Analytical Specificity (Cross Reactivity)	No cross-reactivities were found to:		Prolactin (Reportable Ranges: 50 – 1000 ng/mL)	
Precision	Sample (ng/mL)	Mean (ng/mL)	SD (ng/mL)	CV (%)
Intra-Assay	1 2 3	1.46 12.33 41.87	0.09 0.68 0.97	5.8 5.5 2.3
Inter-Assay	1 2 3	2.95 19.29 36.06	0.27 0.86 1.72	9.0 4.4 4.7
Recovery	Sample (ng/mL)	Measured (ng/mL)	Expected Conc. (ng/mL)	Recovery (%)
	Native (1) + 1.0 + 5.0 + 50	ND 0.996 5.6 49	- 1.0 5.0 50	- 96.0 112.0 98.0
	Native (2) + 1.0 + 5.0 + 50	0.7 1.5 6.6 53	- 1.7 5.7 50.7	- 88.2 115.8 104.5
	Native (3) + 1.0 + 5.0 + 50	1.0 1.7 6.8 48.8	- 2.0 6.0 51	- 85.0 113.3 95.7
Linearity	Sample (ng/mL)	Serial dilution up to	Range (%)	Mean (%)
	6.44 16.60 33.00	1/10 1/10 1/10	89.1 – 96.9 84.9 – 96.0 97.0 – 100.0	92.7 92.2 98.0
High dose hook effect	No High dose hook effect detected.			

16. PRODUCT LITERATURE REFERENCES

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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