ELISA Kit

Catalog # KAQ1081 (96 tests)

Human

HGH

www.invitrogen.com
Invitrogen Corporation
542 Flynn Road, Camarillo, CA 93012
Tel: 800-955-6288
E-mail: techsupport@invitrogen.com

Table of Contents

Contents and Storage

Storage

Store at 2 to 8°C.

Contents

Reagents Provided	Quantity	Color Code	Reconstitution
Microtiter plate with 96 anti-HGH coated wells.	96 wells	blue	Ready to use
Standard 0 μIU/mL in sheep serum with thymol*; lyophilized.	1 vial	yellow	Add 2 mL distilled water
Standards 1-5 μIU/mL in sheep serum with thymol*; lyophilized.	5 vials	yellow	Add 0.5 mL distilled water
Controls 1 and 2 in human serum, thymol*; lyophilized.	2 vials	silver	Add 0.5 mL distilled water
Anti-HGH-HRP Conjugate in stabilizing buffer; 0.2mL per vial.	1 vial	yellow	Dilute 40x with Conjugate Buffer
Conjugate Buffer: Tris-HCl buffer with bovine serum albumin and thymol; 6mL per vial.	1 vial	red	Ready to use
Wash Buffer Concentrate (200x); 10 mL per vial.	1 vial	brown	Dilute 200x with distilled water
Chromogen. TMB (Tetramethylbenzidine); 12 mL per vial.	1 vial	brown	Ready to use
Stop Reagent. 1.0N HCl; 12 mL per vial.	1 vial	white	Ready to use

Note: 1. Standard 0 μIU/mL is recommended for sample dilutions.
2. 1 μIU of the standard preparation is equivalent to 1 μIU of the 2nd IS 98/574.
***See exact values on vial labels.**

Disposal Note

This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Safety

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Introduction

Purpose

The Invitrogen HGH-EASIA is a solid phase Enzyme Amplified Sensitivity Immuno-Assay for the quantitative determination of Human Growth Hormone (HGH) in human serum and plasma.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Principle of the Method

Invitrogen HGH-EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on a microtiter plate.

Standards or samples containing HGH react with capture antibodies (mAbs 1) coated on a plastic well and with monoclonal antibodies (mAbs 2) labeled with horseradish peroxidase (HRP).

After an incubation period allowing the formation of a sandwich: the microtiter plate is washed to remove unbound enzyme labeled antibodies.

The substrate solution (tetramethylbenzidine (TMB) – H_20_2) is added and incubated. The reaction is stopped with H_2S0_4 and the microtiter plate is read at the appropriate wavelength. The amount of substrate turnover is determined colorimetrically by measuring the absorbance which is proportional to the HGH concentration. A standard curve is plotted and HGH concentrations in samples are determined by interpolation from the standard curve.

Background Information

HGH is a polypeptide hormone (molecular weight 21.5 kDa) produced by the acidophil cell of the anterior pituitary under the control of two main substances from the median eminence: Growth-hormone Releasing Factor (GRF) and inhibitory agent, somatostatin. Dopaminergic, adrenergic and serotoninergic neuroendocrine pathways play important roles in the control of HGH secretion. Stimuli of HGH secretion include hypoglycemia, exercise, fasting, meals with a high protein content, deep sleep, stress, glucagon, L DOPA, amino acids, etc. Inhibition of HGH is seen by 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 concentrations vary 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 somatomedin in serum is kept stable by virtue of being largely bound to circulating plasma proteins.

Methods

Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Glass or plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders

Procedural Notes

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. **Do not mix or interchange different reagent lots from various kit lots**.
9. Do not use reagents after the kit expiration date.
10. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
11. In-house controls or kit controls, if provided, should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
13. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.

Directions for Washing

- **Incomplete washing will adversely affect the test outcome**. All washing must be performed with the Wash Buffer provided.
- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- If using an automated washer, follow the washing instructions carefully.

Preparation of Reagents

1. Standards and controls: reconstitute the lyophilized standards and controls with distilled water to the volume specified on the vial label (2 mL for the Standard 0 and 0.5 mL for the other standards and controls). Allow them to remain undisturbed until completely dissolved, then mix well by gentle inversion. Store aliquots at -20°C and avoid repeated freeze/thaw cycles.
2. Wash solution: add 199 volumes of distilled water to 1 volume of wash solution (200x). Use a magnetic stirrer to mix.

Assay Procedure

Be sure to read the *Procedural Notes* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 50 µL of each standard, control or sample into the appropriate wells.
3. Add 50 µL of *Anti-HGH-HRP Conjugate* into all wells.
4. Cover the plate with a plate cover and incubate for **30 minutes at room temperature**.
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 3 times. See **Directions for Washing.**
6. Add 100 µL of *Chromogen* into each well within 15 minutes following the washing step.
7. Incubate the plate for **30 minutes at room temperature and in the dark.** ***Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceeds the limits of the instrument. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
8. Add 100 µL of *Stop Reagent* into each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
9. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Reagent*. Read the plate within 1 hour after adding the *Stop Reagent*.
10. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
11. Read the concentrations for unknown samples and controls from the standard curve. (Samples producing signals greater than that of the highest standard should be diluted in *Standard 0* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

Typical Data (Example)

The following data were obtained for the various standards over the range of 0 to 98 µIU/mL Human HGH.

Standard HGH (µIUmL)	Optical Density (450 nm)
98	2.33
43.5	1.43
12.9	0.50
5.4	0.23
0.45	0.06
0	0.03

Performance Characteristics

Sensitivity

The minimum detectable concentration (MDC) is estimated to be 0.17 µIU/mL and is defined as the concentration corresponding to the mean OD of 20 replicates of the zero standard + 2 standard deviations.

Precision

1. Intra-Assay Precision

Samples of known Hu HGH concentration were assayed in replicates of 20 to determine precision within an assay.

	Sample 1	Sample 2
Mean (µIU/mL)	6.0	16.61
SD	0.34	0.90
%CV	4.9	5.4
SD = Standard Deviation CV = Coefficient of Variation		

2. Inter-Assay Precision

Samples were assayed 8 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2
Mean (µIU/mL)	11.4	23.5
SD	0.9	1.2
%CV	8.1	5.1
SD = Standard Deviation CV = Coefficient of Variation		

Linearity of Dilution

Sample	Serum dilution	Theoretical conc. (µIU/mL)	Measured conc. (µIU/mL)
Serum	1/2	-	97.7
	1/4	48.8	57.0
	1/8	24.4	27.7
	1/16	12.2	13.6
	1/32	6.1	6.4
	1/64	3.1	3.0
Plasma	1/2	-	12.8
	1/4	6.4	6.9
	1/8	3.2	3.3
	1/16	1.6	1.8
	1/32	0.8	0.8

Samples were diluted with Standard 0.

Recovery

Sample	Added HGH (µIU/mL)	Recovery (µIU/mL)	Recovery (%)
Serum	52.1	58.9	113
	26.8	28.1	105
	13.5	12.8	95
	4.3	4.1	95
Plasma	52.1	53.0	102
	26.8	25.3	94
	13.5	13.2	98
	4.3	4.6	106

Samples were diluted with Standard 0.

Specificity

The cross-reactivities of HCG, HPL, and PRL were determined by addition of each analyte to serum samples respectively containing 1.9 µIU/mL and 13.5 µIU/mL of HGH and measuring the apparent HGH concentration. As shown below, the cross-reactions with HCG, HPL and PRL are insignificant.

Added analyte to a low HGH value serum (1.9 µIU/mL)	Observed HGH value (µIU/mL)	Added analyte to a high HGH value serum (13.5 µIU/mL)	Observed HGH value (µLU/mL)
-	1.9		13.5
HCG (100,000 mIU/mL)	2.0	HCG (100,000 mIU/mL)	14.3
HPL (10,000 ng/mL)	1.4	HPL (10,000 ng/mL)	13.0
PRL (12,500 ng/mL)	1.8	PRL (12,500 ng/mL)	12.6

High Dose Hook Effect

A sample spiked with HGH up to 4,000 µIU/mL gives a result higher than the last standard point.

Limitations of the Procedure

Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point with *Standard 0*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu HGH in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Appendix

Troubleshooting Guide

Elevated background	*Cause:* Insufficient washing and/or draining of wells after washing. Solution containing HRP-conjugate can elevate the background if residual is left in the well. *Solution:* Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid. *Cause:* Contamination of substrate solution with metal ions or oxidizing reagents. *Solution:* Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil. *Cause:* Contamination of pipette, dispensing reservoir or substrate solution with SAV-HRP conjugate. *Solution:* Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen. *Cause:* Incubation time is too long or incubation temperature is too high. *Solution:* Reduce incubation time and/or temperature.
Elevated sample/ standard ODs	*Cause*: Incubation times extended. *Solution:* Follow incubation times outlined in protocol. *Cause:* Incubations carried out at 37°C when RT is dictated. *Solution:* Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.
Poor standard curve	*Cause:* Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted. *Solution:* NEVER substitute any components from another kit. *Cause*: Errors in pipetting the standard or subsequent steps. *Solution*: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

Weak/no color develops	*Cause:* Reagents not at RT (25 ± 2°C) at start of assay. *Solution:* Allow ALL reagents to warm to RT prior to commencing assay. *Cause:* Incorrect storage of components, e.g., not stored at 2 to 8°C. *Solution:* Store all components exactly as directed in protocol and on labels. *Cause:* Working HRP-conjugate solution made up longer than 15 minutes before use in assay. *Solution:* Use the diluted HRP-conjugate within 15 minutes of dilution. *Cause:* TMB solution lost activity. *Solution 1:* The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded. *Solution 2:* Avoid contact of the TMB solution with items containing metal ions. *Cause:* Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized. *Solution:* Please contact Technical Support for advice when using nonvalidated sample types. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.
Poor Precision	*Cause:* Errors in pipetting the standards, samples or subsequent steps. *Solution:* Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip. *Cause:* Repetitive use of tips for several samples or different reagents. *Solution:* Use fresh tips for each sample or reagent transfer. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.

Technical Support

Contact Us

For more troubleshooting tips, information, or assistance, please call, email, or go online to www.invitrogen.com/ELISA.

USA:

Invitrogen Corporation
542 Flynn Road
Camarillo, CA 93012

Tel: 800-955-6288

E-mail: techsupport@invitrogen.com

Europe:

Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK

Tel: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6117

E-mail: eurotech@invitrogen.com

References

1. Camanni, F., et al. (1977). Effect of dopamine on plasma growth hormone and prolactin levels in normal and acromegalic subjects. *J. Clin. Endo. Metab*. 44:465.
2. Daughaday, W.H. (1981). The adenohypophysis in: "Textbook of Endocrinology," R.H. Williams ed., W.B. Saunders. Philadelphia, p. 73.
3. Drobny, E.C., Amburn, K., Baumann, G. (1983). Circadian variation of basal plasma growth hormone in man. *J. Clin. Endo. Metab*. 57:524-528.
4. Hashida, S., et al. (1985). Basal level of human growth hormone hGH in normal serum. *Clin. Chem. Acta* 151:185-186.
5. Lewis, V.J. et al. (1980). Human growth hormone: A complex of proteins. *Rec. Progr. Horm*. *Res.* 36:488.
6. Li, C.H. (1974). Human growth hormone: perspectives on its chemistry and physiology. In: "Advances in human growth hormone research," S. Raiti ed. U.S. Department of Health, Education and Welfare Publ. DMEW publ. No. (NJH) 74-612, p. 321.
7. Reutens, A.T. (1995). Evaluation and application of a highly sensitive assay for serum growth hormone (GH) in the study of adult GH deficiency. *J. Clin. Endocrinol. Metab*., 80(2):480-485.
8. Crowley, S., et al. (1995). Growth and the growth hormone axis in prepubertal children with asthma. *J. Pediatr*. 126(2):297-303.
9. Kelly, J.T., et al. (1993). Effect of different oral oestrogen formulations on insulin-like growth factor I, growth hormone and growth hormone binding protein in post-menopausal women. *Clin. Endocrinol*. 39(5):561-567.

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Support Representatives. Invitrogen warrants that all of its products will perform according to the specifications stated on the Certificate of Analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order. Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives. **Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.**

Licensing Information

These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

NOTES

Human HGH Assay Summary

Assay Summary		
	Standards (µL)	Controls-samples (µL)
Standards (0-5) Controls-samples Anti-HGH-HRP Conjugate	50 - 50	- 50 50
Incubate for 30 minutes at RT. Aspirate the content of each well. Wash 3 times with 0.4 mL of wash solution and aspirate.		
Chromogen (TMB)	100	100
Incubate for 30 minutes at RT		
Stop Reagent	100	100
Read the absorbance of each well at 450 nm and calculate results.		