REF IS-3700	IDS-iSYS Human Growth Hormone (hGH)	
Instructions for Use	*IN VITRO DIAGNOSTIC*	CE

Intended Use

For In Vitro Diagnostic Use

The IDS-iSYS Human Growth Hormone Assay (IDS-iSYS hGH) is intended for the quantitative determination of Human Growth Hormone (hGH) in human serum or plasma on the IDS-iSYS Multi-Discipline Automated Analyser (Analyser). Results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the assessment of growth disorders.

Summary and Explanation

Growth hormone (hGH) is a polypeptide hormone secreted from the acidophil cells of the anterior pituitary gland. Secretion is episodic and is associated with exercise, the onset of deep sleep or post-prandially in response to falling glucose levels. Synthesis and release are under the control of hypothalamic releasing peptides and inhibitory peptides such as somatostatin. More recently, a gastric peptide, Ghrelin, has been shown to also stimulate hGH secretion. In contrast, the mediator of many hGH actions in the periphery, insulin-like growth-factor I (IGF-I) exerts an inhibitory effect through negative feedback mechanisms[1]. hGH in circulation consists of several molecular isoforms, with 22,000 Dalton hGH being the most abundant, followed by a 20,000 Dalton hGH variant produced by alternative splicing. Approximately 50% of circulating hGH is bound to a high affinity binding protein[2].

hGH is physiologically important in two main areas. Firstly, it has an integral role in skeletal growth which is well demonstrated in either excess or deficiency in childhood. The action of hGH in part is mediated through IGF-I as well as promoting protein synthesis and the uptake of amino acids into cells. Secondly, hGH influences intermediary metabolism by stimulating lipolysis and is antagonistic to the insulin-mediated uptake of glucose[3]. hGH secretion is stimulated by hypoglycaemia and suppressed by hyperglycaemia.

In childhood, symptoms of hGH deficiency are retarded growth and dwarfism. Aetiology is often unknown and an absolute or relative deficiency usually becomes apparent at about 2 years of age. Diagnosis can be confirmed by demonstrating low serum hGH which does not respond to stimulation tests. hGH deficiency is a major cause of severe short stature and diagnosis at an early stage is essential for successful therapy[4]. Hyposecretion in adults usually becomes apparent during the laboratory investigation of hypopituitarism[5,6].

Hypersecretion, commonly due to adenoma of the acidophil cells, is characterised by two conditions depending on whether it becomes apparent before or after fusion of the bony epiphyses. In childhood, excess hGH is characterised by gigantism. Heights of 8 feet (2.4 metres) may be achieved and may also be associated with hypogonadism. In adults acromegaly results, a condition characterised by progressive thickening of bone and soft tissue. Diagnosis is usually confirmed by dynamic function testing which demonstrates a raised serum hGH level which does not fall in response to an oral glucose load[7]. In conditions where there are nutritional disturbances such as anorexia, starvation, renal failure and hepatic cirrhosis, increased basal hGH levels may be found.

Recombinant hGH is available for treatment of hGH deficiency in both children and adults[4,5,6]. hGH excess is treated by surgery, irradiation therapy or somatostatin analogues[8]. More recently, a hGH receptor antagonist has been developed, which shares structural homology to hGH and competes with hGH for binding to the hGH receptor[9].

Method Description

The assay is based on chemiluminescence technology. Samples are incubated with a biotinylated anti-hGH monoclonal antibody and streptavidin labelled magnetic particles. The magnetic particles are “captured” using a magnet and a wash step performed. An acridinium labelled anti-hGH monoclonal is added and following a further incubation step a second wash step is performed. Trigger reagents are added and the resulting light emitted by the acridinium label is directly proportional to the concentration of hGH in the original sample.

Warnings and Precautions

The IDS-iSYS hGH is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in these Instructions for Use (IFU). IDS Limited will not be held responsible for any loss or damage (except as required by statute), howsoever caused arising out of non-compliance with the instructions provided.

CAUTION: This kit contains material of animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Appropriate precautions and good laboratory practice must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Sodium Azide

Some reagents in this kit contain sodium azide < 0.1% (w/w) which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

Handling Precautions

The reagents provided in the kit are ready to use.
Before a new cartridge is loaded onto the Analyser, the magnetic particle container requires mixing by the operator with a brisk rotation motion. This will resuspend the magnetic particles that have settled during shipment. It is very important to avoid any foam formation.

Shelf Life and Storage of Reagents

Prior to first use, store the cartridge and the calibrators in an **upright** position in the dark at 2 to 8°C. Do not freeze the cartridge or the calibrators.

Reagent shelf life	Cartridge	Calibrators
Before opening at 2 - 8°C	To the expiry date	
After opening at 2 - 8°C	28 Days	To the expiry date
On board the Analyser **(*)**	11 Days	3 hours

(*) Continuous on board stability.

Sample Collection and Storage

The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (sodium citrate, lithium heparin, sodium heparin or ammonium heparin) samples. Samples should be

separated as soon as possible after collection. EDTA plasma cannot be used with the IDS-iSYS hGH assay.
Store samples at -20°C. Avoid repeated freeze-thaw of samples.
Samples containing particulate matter must be centrifuged before performing the assay.
Do not use heat-inactivated samples.
To minimise possible evaporation effects, samples, calibrators, and controls should be measured within 3 hours after being placed on the analyser.
Before assaying, make sure that samples, calibrators and controls are at room temperature (20 - 25°C).
Note: Some sample collection tubes that are commercially available might affect the results of testing in particular cases.
It is recommended to follow the instructions of the tube manufacturer especially when processing samples in primary tubes.

Procedure

Materials Provided

Reagent Cartridge
Note: Bottle 4 is deliberately empty and is not used.

MPS3
Magnetic particles coated with streptavidin in phosphate BSA buffer with sodium azide as preservative (< 0.1%). 1 bottle, 2.3 mL.

CONJ
Anti-hGH monoclonal antibody labelled with an acridinium ester derivative, in buffer containing BSA, mouse serum and horse serum with sodium azide as preservative (< 0.1%). 1 bottle, 23 mL.

Ab-BIOT
Anti-hGH monoclonal antibody labelled with biotin, in buffer containing BSA and mouse proteins with sodium azide as preservative (< 0.1%). 1 bottle, 11.5 mL.

Calibrators
CAL A
CAL B
Horse serum containing hGH and sodium azide as preservative (< 0.1%), 1 each of 2 concentration levels, 2 mL.

Mini CD
Contains IFU for IDS-iSYS reagents and CRY files.

Materials Required But Not Provided

IDS-iSYS Multi-Discipline Automated Analyser : IS-310400
Bio-Rad Lyphochek® Immunoassay Plus Control, Cat. No. 370 (Trilevel, 12 x 5 mL) **or** Cat. No. 371 for Level 1 (12 x 5 mL) and Cat. No. 372 for Level 2 (12 x 5 mL) and Cat. No. 373 for Level 3 (12 x 5 mL) **or** Cat. No. 370X (Trilevel MiniPak 3 x 5 mL).
IDS-iSYS Cuvettes Cube: IS-CC100, box of 960 cuvettes.
IDS-iSYS System Liquid: IS-CS100, 5 L container, ready to use.
IDS-iSYS Wash Solution: IS-CW100, 10 L container, ready to use.
IDS-iSYS Triggers Set A and B: IS-CT100, 2 x 250 mL per bottle, ready to use.
IDS-iSYS Cartridge Check System: IS-6010, ready to use.
IDS-iSYS Sample Cups (500 µL): IS-SC105.

Assay Procedure

Reagent Cartridge
The reagents provided in the cartridge are ready to use. The analyser automatically performs the mixing of magnetic particles to maintain homogeneity. Before a new cartridge is loaded on board the analyser, mix the magnetic particles container by brisk rotation motion. Avoid foam formation.
The barcode is read when the cartridge is loaded on the reagent tray. If the label cannot be read by the analyser barcode reader, a manual procedure exists to enter the barcode data (see the IDS-iSYS User Manual).
Load the cartridge on the reagent tray and wait for at least 40 minutes before starting the assay.
If the cartridge is removed from the reagent tray, store the cartridge vertically at 2 - 8°C in the dark.

Calibrators
The hGH calibrators are ready to use. Leave the calibrators at room temperature for 10 minutes and gently mix the bottles by hand. Avoid formation of foam. Pipette approximately 300 µL of calibrators into sample cups and place on the machine. Discard the material in the sample cups after use. DO NOT return material to the calibrator bottle.

Analyser Calibration
The two hGH calibrators are required to perform the adjustment of the master curve. The calibrators are supplied with the kit and calibrators from another lot must not be used.

Note that to perform a master curve adjustment, controls MUST be run at the same time as the calibrators.

All data required for the calibration of the cartridge batch can be found on the mini CD. Use calibrator levels A and B to adjust the master curve to the reagents on board the Analyser. Check for the presence of a hGH cartridge on the reagent tray and the availability of the cartridge master curve in the database. If the data for the lot of calibrators is not available on board the analyser, load the data using the mini CD provided with the calibrator.

Start the immunoassay calibration on the IDS-iSYS Analyser according to the IDS-iSYS User Manual. The calibration is carried out in triplicate. RLU CVs of > 5% will result in a failed calibration. One replicate may be removed to meet the calibration requirements. As stated above, please note that controls must also be run. Verify and approve the calibration according to the calibration status displayed in the calibration windows and discard the calibrator from the sample tray after use.

Calibration
The IDS-iSYS hGH assay is calibrated to the WHO International Standard for Somatropin from NIBSC, code 98/574.

Calibration Frequency
A new calibration is required:

- Each time a new lot of cartridges is loaded on board.
- Each time a new lot of trigger or cuvettes is used.
- When the control values do not fall within the defined ranges.
- When the calibration has expired.

- After Analyser service.

Verification of the calibration is automatic and managed by the Analyser.

Quality Control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results.
Use Bio-Rad Lyphochek® Immunoassay Plus Control for quality control.
To ensure validity of results at least three controls with varying levels of hGH should be measured. Other suitable control material can be used in addition to the Bio-Rad Lyphochek® Immunoassay Plus Control. Controls should be tested at (or near) the beginning of every run containing patient samples and also during calibrations or according to local regulations. It is recommended that the controls be routinely run in duplicate. Laboratories should test controls at least once per shift.

Entering Control Targets into the IDS-iSYS Software
Open the IDS-iSYS software and in the Management Of Lots drop down menu select Control.
Select the control level that you wish to enter the value for and click the New Lot button.
In the Identification window enter the lot number and expiry of the control level in their respective fields.
In the Analytes window enter the supplied Target Value and Deviation.
Click Validate to confirm.
Repeat the process for the other control levels.

Preparation, Handling and Storage of Controls
Refer to the Bio-Rad Lyphochek® Immunoassay Plus Control Instructions For Use for preparation, handling and storage instructions.

Stability of Controls Onboard
Controls are stable on board the analyser for up to 3 hours (continuous on board stability).

When using the Controls
Pipette approximately 300 µL of each control into sample cups and place on the machine. Discard the material in the sample cups after use. DO NOT return material to the control bottles.

Controls should be run as duplicate determinations at least once every 24 hours when the test is in use and during every calibration. The control values must be within the acceptable ranges specified. If a control is out of its specified range, the associated test results are invalid and samples must be retested. Recalibration may be required.

Determination of Sample hGH levels

Process samples according to the IDS-iSYS User Manual.

Calculation of Results

The hGH concentration of each sample is calculated automatically. The display of the concentrations (screen or printed) is produced upon user request.
The IDS-iSYS hGH Assay uses a 4-parameter logistic curve fit (4PL) to calculate the hGH concentrations.

Conversion of Units:

$$X \text{ ng/mL} \xrightarrow{\times 3.00} Y \text{ µIU/mL}$$
$$Y \text{ µIU/mL} \xrightarrow{\times 0.333} X \text{ ng/mL}$$

Measurement Range (Reportable Range)

The reportable range of the assay is 0.050-100 ng/mL. Any value that reads below 0.050 ng/mL should be reported as "< 0.050 ng/mL".

Dilution

Samples with hGH concentrations above the reportable range should be diluted manually with a low concentration human serum sample in a ratio of 1 in 2. The results for diluted samples must be multiplied by the dilution factor 2 and corrected for the concentration of the low sample.

Limitations of Use

1. As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
2. The performance characteristics of this assay have not been established in a paediatric population.
3. The following substances do not interfere in the IDS-iSYS hGH Assay when the concentrations presented in the following table are below the stated threshold.

Potentially Interfering Agent	Threshold Concentration
Lipid	3000 mg/dL
Bilirubin	200 mg/L
Haemoglobin	500 mg/dL
Biotin	300 nmol/L
Growth Hormone Binding Protein (GHBP)	140 ng/mL

4. The hook effect was tested using concentrations of hGH up to 15,000 ng/mL. No hook effect was observed.

Expected Values

Each laboratory should determine ranges for their local population.
Growth Hormone (GH) is secreted from the anterior pituitary gland in a pulsatile or episodic manner and has a short half-life. Regulation of GH level in blood is influenced by a number of hormonal and physiological factors. Normal serum GH levels average less than 5 ng/mL and single measurements of GH are generally low in subjects whose blood is drawn while they are awake. For these reasons, a single measurement of GH does not provide adequate information for evaluating GH adequacy. Even among normal healthy individuals, a single fasting sample is highly variable and is not considered a useful measure of GH. Stimulation tests are commonly employed with baseline and post stimulation blood sampling and normal baseline levels are generally less than 10 ng/mL.

Basal GH, Normal Adults < 10 ng/mL (n=150)

Performance Data

Representative performance data are shown. Results obtained at individual laboratories may vary.

Sensitivity
The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" using 60 blanks and at least 120 low level samples.

LoB	0.005 ng/mL
LoD	0.005 ng/mL
LoQ	0.049 ng/mL

Precision

Precision was evaluated in accordance with a protocol based on CLSI EP-5A2, "Evaluation of Precision Performance of Quantitative Measurement Methods". Three serum controls were assayed using three lots of reagents in duplicate twice per day for 20 days on two instruments.

Control	n	mean	Within-run		Total	
		(ng/mL)	SD	CV%	SD	CV%
1	40	1.49	0.03	1.8%	0.16	10.4%
2	40	9.87	0.35	3.5%	0.88	8.9%
3	40	25.23	0.55	2.2%	1.48	5.9%

Recovery

Recovery was assessed by adding intact hGH to samples prior to assay.

Sample Conc ng/mL	hGH added ng/mL	Measured ng/mL	Recovery ng/mL	Recovery %
0.58	12.50	13.03	12.45	100%
0.58	25.00	26.05	25.47	102%
0.58	37.50	39.43	38.85	104%
0.58	50.00	50.91	50.33	101%
0.53	12.50	13.23	12.70	102%
0.53	25.00	25.82	25.29	101%
0.53	37.50	39.15	38.62	103%
0.53	50.00	50.52	49.99	100%
			Mean	**102%**

Linearity

Linearity was evaluated based on CLSI EP-6A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". Samples containing varying concentrations of hGH were assayed in duplicate. The resulting mean concentrations were compared to predicted concentrations. Samples were prepared by diluting a high patient sample with a low patient sample prior to assay.

Predicted Concentration (ng/mL)	Measured Concentration (ng/mL)	Variation	
		(ng/mL)	%
< 0.05	0.24		
15.68	14.77	-0.91	-6%
31.44	30.80	-0.64	-2%
47.19	47.55	0.36	1%
62.95	64.40	1.45	2%
78.70	79.95	1.25	2%
94.46	92.64	-1.82	-2%

Predicted Concentration (ng/mL)	Measured Concentration (ng/mL)	Variation	
		(ng/mL)	%
0.43	0.39	-0.04	-9%
2.59	2.63	0.04	2%
4.75	4.83	0.08	2%
6.90	6.79	-0.11	-2%
9.06	9.05	-0.01	0%
11.21	11.28	0.07	1%
13.37	13.34	-0.03	0%

Method Comparison

The IDS-iSYS hGH Assay was compared against a recognised hGH assay (X) for the quantitative determination of hGH, following CLSI EP-9A2, "Method Comparison and Bias Estimation Using Patient Samples". A total of 49 samples, selected to represent a wide range of hGH concentrations [0.11-19.12 ng/mL], were assayed by each method. Linear regression analysis was performed on the comparative data:
IDS-iSYS = 0.95 (X) + 0.05 (95% CI of the slope and intercept were 0.90 to 1.01, and -0.22 to 0.32, respectively); correlation coefficient (r) = 0.98.

Specificity

The specificity was assessed with the following analytes.

Analyte	Cross-Reactivity
hGH 22kDa	100%
hGH 20kDa (10 ng/mL)	ND*
Placental hGH (200 ng/mL)	ND*
HPL (10,000 ng/mL)	ND*
Prolactin (40,000 ng/mL)	ND*
Pegvisomant (50,000 ng/mL)	ND*

* Not detectable.

Bibliography

1. Giustina A, Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev. 1998 Dec; 19(6):717-97.
2. Baumann G. Growth hormone heterogeneity: genes, isohormones, variants, and binding proteins. Endocr Rev. 1991 Nov; 12(4):424-49.
3. Møller N, Jørgensen JO. Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. Endocr Rev. 2009 Apr; 30(2):152-77.
4. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, Chernausek SD, Savage MO, Wit JM; 2007 ISS Consensus Workshop participants. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. J Clin Endocrinol Metab. 2008 Nov; 93(11):4210-7.
5. Ho KK; 2007 GH Deficiency Consensus Workshop Participants. Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric

Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. Eur J Endocrinol. 2007 Dec; 157(6):695-700.

6. Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Shalet SM, Vance ML; Endocrine Society's Clinical Guidelines Subcommittee, Stephens PA. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2006 May; 91(5):1621-34.
7. Growth Hormone Research Society; Pituitary Society. Biochemical assessment and long-term monitoring in patients with acromegaly: statement from a joint consensus conference of the Growth Hormone Research Society and the Pituitary Society. J Clin Endocrinol Metab. 2004 Jul; 89(7):3099-102.
8. Consensus statement: medical management of acromegaly. Melmed S, Casanueva F, Cavagnini F, Chanson P, Frohman LA, Gaillard R, Ghigo E, Ho K, Jaquet P, Kleinberg D, Lamberts S, Laws E, Lombardi G, Sheppard MC, Thorner M, Vance ML, Wass JA, Giustina A. Eur J Endocrinol. 2005 Dec; 153(6):737-40.
9. Kopchick JJ, Parkinson C, Stevens EC, Trainer PJ. Growth hormone receptor antagonists: discovery, development, and use in patients with acromegaly. Endocr Rev. 2002 Oct; 23(5):623-46.

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