hGH [I-125] IRMA KIT

(REF: RK-5CT)

The ^{125}I-hGH IRMA system provides a direct quantitative *in vitro* determination of human Growth Hormone (hGH) in human serum. hGH can be assayed in the range of 0.19-100 µIU/ml using 50 µl serum samples.

Introduction

The human Growth Hormone (Somatotropin, hGH) is a protein hormone secreted by the pituitary gland. hGH is a heterogeneous protein composed of several molecular isoforms. The isoform with a molecular mass of 22 kDa is the most abundant, representing more than 90 % of the circulating hGH. The structure of the molecule is similar to that of Prolactin (hPRL) and Placental Lactogen (hPL).

The secretion of hGH is under the double hypothalamic regulation of growth hormone-releasing hormone (GH-RH) and an inhibitory agent, somatostatin.

Several effects of hGH have been known. It does not only regulate protein synthesis, the growth of the skeleton, the muscles and the viscera but has also lipolytic and lactogenic effects and influences glycogen storage in the liver as well. In clinical practice, the diabetogenic effect of hGH has also been well-known.

hGH mean serum level progressively decreases after birth. Its level increases again significantly during puberty to further decrease with aging. Because of the pulsative nature of hGH secretion, a single measurement of hGH does not reflect the endogenous hGH secretion. About 50% of the population has a very low, sometimes undetectable hGH concentration. Standardized stimulatory tests are therefore necessary to assess pituitary hGH secretion.

The measuring of hGH can widely be used in clinical practice for evaluating the hypo- and hypersecretion related to growth retardation, hypopituitarism, acromegaly and other diseases.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.

The ^{125}I labelled signal-antibody binds to an epitope of the GH molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During a 2-hour incubation period with shaking immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of hGH, the unknown concentration of hGH in patient samples can determined.

Contents of the kit

1) 1 bottle of TRACER (21 ml), ready to use, containing about 740 kBq ^{125}I-anti-hGH and biotinylated anti-hGH monoclonal antibodies in buffer with red dye and 0.1 % NaN_3.
2) 6 vials of STANDARDS (6 x 1ml) lyophilized, containing app. 0, 0.3, 1.3, 5.5, 23, 100 µIU/ml hGH (calibrated against WHO 2nd IS 98/574) in bovine serum with 0.1% NaN_3. The exact concentration of the standards is specified on the labels and in the quality certificate enclosed See *Preparation of reagents.*
3) 1 vial of CONTROL SERUM. Lyophilized human serum with 0.1% NaN_3. The concentration of the control serum is specified in the quality certificate enclosed. See *Preparation of reagents*.
4) 2 boxes of COATED TUBE, Ready to use.
5) 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.
6) 1 bottle of WASH BUFFER CONCENTRATE (20 ml), containing 0.2% NaN_3. See *Preparation of reagents.*

Quality certificate

Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (50, 200 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, absorbent tissue, gamma counter

Recommended tools and equipment
Repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-ml pipette)

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise stored deep frozen (-20°C) up to 4 months. Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided. Do not use lipemic, haemolyzed or turbid specimens. Samples with a hGH concentration higher than that of the most concentrated standard should be diluted and re-assayed.

Preparation of reagents, storage

Add the wash buffer concentrate (20 ml) to 700 ml distilled water to obtain 720 ml wash solution. Upon dilution, store at 2-8°C until expiry date of the kit.

Add 1000 µl distilled water to the lyophilized standards and control serum. Mix gently with shaking or vortexing (foaming should be avoided). Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes. Store at -20°C until expiry date of the kit.

Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.

CAUTION!
Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide, refer to Table 1.)

1. Equilibrate reagents and samples to room temperature before use.
2. Label coated tubes in duplicate for each standard (S1-S6), control serum and samples. Optionally, label two test tubes for total counts (T).
3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Pipette 50 µl of standards, control and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
5. Pipette 200 µl of tracer into each tube.
6. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube.
7. Incubate tubes for 2 hours, shaking at room temperature.

8. Add 2.0 ml of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside-down position place the rack on an absorbent paper for 2 minutes.
9. Return the tube-rack to an upright position, and repeat step-8 two more times.
10. Count each tube for at least 60 seconds in a gamma counter.
11. Calculate the GH concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microliters)

Tubes	Total	Standard	Control	Sample
Standard		**50**		
Control			**50**	
Sample				**50**
Tracer	**200**	**200**	**200**	**200**
Shake for 2 hours at room temperature				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Repeat twice washing step				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (CPM) for each pair of assay tubes.

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T\ (\%) = \frac{S_{2\text{-}6} / C / M_x\ (cpm) - S_1\ (cpm)}{T(cpm)} \times 100$$

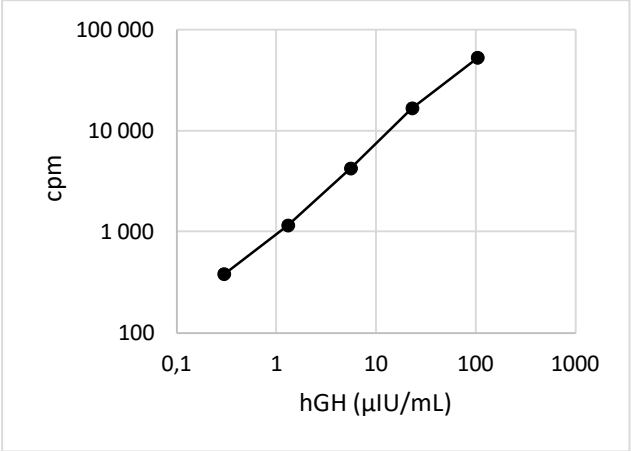
Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of hGH.

Determine the hGH concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Table 2. Typical assay data

Tubes	hGH (µIU/mL)	Mean cpm (n = 20)	B/T%	hGH (µIU/mL)
T		147326		
S1	0	145	0.10	
S2	0.3	381	0.26	
S3	1.3	1150	0.78	
S4	5.6	4263	2.89	
S5	23	16630	11.29	
S6	105	53125	36.06	
C		4465	3.03	6.07



Characterization of assay

Conversion of units: ng/mL = µIU/mL x 0.33

Sensitivity

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidelines in CLSI document EP17-A2.

Limit of Blank (LoB): 0.04 µIU/ml

Limit of Detection (LoD): 0.09 µIU/ml

Limit of Quantitation (LoQ): 0.19 µIU/ml

Specificity

Cross reactivity with hPL is 0.2% (declared by the antibody manufacturer).

No cross reactivity with the following hormones was detected for concentrations higher than physiological values, up to the following concentrations:

Hormone	Concentration
hTSH	412 µIU/mL
hLH	404 mIU/mL
hFSH	441.5 mIU/mL
hCG	310 IU/mL
hPRL	316 ng/mL

Precision and reproducibility

Single-site precision

Single-site precision was calculated using 5 serum pools at different hGH concentrations, according to CLSI document EP05-A3. Samples were measured in twenty testing days, two runs per day and using two replicates per run.

Sample	Mean (µIU/mL)	*Repeatability*		*Within-laboratory Precision*	
		SD	CV%	SD	CV%
Pool 1	1.00	0.03	3.41	0.04	4.18
Pool 2	3.47	0.07	2.10	0.10	3.03
Pool 3	8.86	0.11	1.24	0.17	1.86
Pool 4	21.32	0.32	1.50	0.48	2.25
Pool 5	65.64	0.68	1.03	1.53	2.34

Multisite precision

Multisite precision was calculated using 5 serum pools at different hGH concentrations, according to CLSI document EP05-A3. Samples were measured in three different sites, at each site five runs were performed, one run per day and using five replicates per run.

Sample	Mean (µIU/mL)	*Repeatability*		*Within-laboratory Precision*		*Reproducibility*	
		SD	CV%	SD	CV%	SD	CV%
Pool 1	0.95	0.04	3.81	0.05	4.93	0.05	4.95
Pool 2	3.32	0.14	4.06	0.20	6.00	0.21	6.20
Pool 3	8.42	0.25	2.92	0.34	4.07	0.35	4.20
Pool 4	22.16	0.75	3.37	0.95	4.28	0.97	4.38
Pool 5	68.54	2.04	2.97	2.54	3.71	2.69	3.93

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of hGH. The average per cent recovery for nine human serum samples spiked with hGH at 3 levels was 99.6%. The lowest result was 92.23% and the highest result was 104.37%.

Linearity

The linearity was evaluated according to CLSI EP06-A guideline, using the polynomial method. The method was found linear from 0.03 µIU/mL to 116.1 µIU/mL, within 10% error in this interval.

Hook effect

There is no high dose "hook effect" up to a hGH concentration of 5000 µIU/ml.

Interference:

Interference testing was performed according to CLSI document EP7-A2. Predefined acceptable interference threshold: <15%. Interference could not be detected for endogenous substances and drugs up to the following concentrations:

Bilirubin	684 µmol/L
Triglycerides	16.94 mmol/L
Haemoglobin	10 g/L
Rheumatoid Factor	400 IU/mL
Biotin	200 ng/mL
Acetaminophen	15.6 mg/dL
Acetylsalicylic acid	3.0 mg/dL
Ascorbic acid	5.25 mg/dL
Diclofenac	2.4 mg/dL
Ibuprofen	21.9 mg/dL
Bromocriptine	10 mg/dL
Cabergoline	1.0 mg/dL
Lanreotide	10 mg/dL
Octreotide	1.2 mg/dL

Expected Values

Given the variability of hGH secretion within a single individual and between different individuals, the basal test is considered of limited diagnostic significance and assessments based on the response of hGH to challenge tests are to be preferred. Basal reference values are to be considered merely indicative.

It is recommended that each laboratory determine a reference range for healthy persons for its own patient population, since this may vary in different laboratories or regions.

Reference Interval was established following the EP28-A3c CLSI Guideline, using the non-parametric method. 282 presumably healthy blood donors were evaluated.

Values are expressed in µIU/mL. *ND = lower than LoD*

Unit conversion: ng/mL = µIU/mL x 0.33

	Female (n = 141)		Male (n = 141)	
	2.5%	97.5%	2.5%	97.5%
Central 95% reference limits	0.09	30.0	ND	6.1
90% confidence intervals	ND-0.11	22.3-45.9	ND	3.1-8.7

Due to the small sample size, pediatric ranges are informative only:

Boys				
Age group	samples	median	minimum measured	maximum measured
3-4 y	40	1.86	0.19	16.0
5-6 y	40	0.87	0.16	23.6
7-8 y	37	0.75	0.08	38.7
9-10 y	35	0.64	0.04	31.5
11-12 y	34	0.47	0.07	19.1
13-14 y	31	0.78	0.12	38.7
15-16 y	28	0.39	0.13	28.7
17-18 y	31	0.37	0.08	38.7

Boys prepubertal age (3-14 y)			**Boys pubertal age (15-18 y)**		
Samples	Median	97% range	Samples	Median	97% range
217	0.84	ND - 28	59	0.37	ND - 28

Girls				
Age group	samples	median	minimum measured	maximum measured
3-4 y	27	1.98	0.33	17.5
5-6 y	30	1.3	0.19	28.0
7-8 y	33	0.66	0.05	20.7
9-10 y	34	0.71	0.1	17.2
11-12 y	36	1.03	0.18	36.7
13-14 y	39	0.68	0.16	21.6
15-16 y	41	3.65	0.15	41.2
17-18 y	37	3.56	0.19	41.5

Girls prepubertal age (3-14 y)			**Girls pubertal age (15-18 y)**		
Samples	Median	97% range	Samples	Median	97% range
199	0.95	ND - 20	78	3.61	0.15 - 40

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with

open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Addition of wash buffer.** For the addition of wash buffer, the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precaution

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of Human Immunodeficiency Virus antibody (Anti-HIV-1), Hepatitis B surface Antigen (HBsAg) and Treponema antibody.

Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.

Bovine components originate from countries where bovine spongiform encephalopathy has not been reported. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard

Some components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 68 mg.

MSDS: Safety data sheet is publicly available on website www.izotop.hu/immunoassay.

Storage and shelf life

Store this product at a temperature of 2-8°C. At this temperature each reagent is stable until the expiry date indicated on the kit label and in the Certificate of Analysis.

The expiry date printed on the vials is exclusively valid for the long-term storage of components by the manufacturer, the user should not take it into account.

After opening the kit, follow storage conditions indicated in paragraph Preparation of reagents, storage.

Shelf-life: 60 days from availability.

Literature

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Symbols key

	Use by	CONTROL	Control
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	WASHB	Wash buffer
IVD	In vitro diagnostic medical device		Temperature limitation Store between 2-8°C
	Manufacturer		Radioactive Material
REF	Catalogue number		CE

Website: http://www.izotop.hu
Technical e-mail: immuno@izotop.hu
Commercial e-mail: commerce@izotop.hu

INSTITUTE OF ISOTOPES Ltd.
1535 Budapest. Pf.: 851.
Tel.: (36-1)392-2577, Fax: (36-1)395-9247

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