Kit for the radioimmunometric assay for a direct quantitative determination of human growth hormone (hGH) in serum.

For In Vitro Diagnostic use

Kit content:
- Coated tubes 2 x 50
- Tracer ≤ 308 kBq 1 x 32 mL
- Calibrator 0 1 x 2 mL
- Calibrators 1-5 5 x 0.5 mL
- Control 1 x 0.5 mL
- Tween 20 1 x 10 mL
- Plastic bag 1
- Instruction For Use 1

Warning: Some reagents contain sodium azide
1. INTENDED USE

HGH-US is a radioimmunoassay for the for a direct quantitative determination of human growth hormone (hGH) in serum. hGH measurement may be indicated for:
- Exploration of growth disorders
- Monitoring of treatment by recombinant hGH
- Follow-up of acromegaly treatment

2. INTRODUCTION

The human Growth Hormone (hGH) is a polypeptidic hormone consisting of 191 amino acids linked by two disulphide bonds, with a molecular weight of 22kD. It is synthesized by the somatotropic cells of the anterior pituitary in the form of a precursor or pre-hormone whose proteolytic cleavage gives rise to the active hormone. Its pulsatile secretion is controlled by the hypothalamus (neurotransmitters) and by physiological stimuli (stress, sleep, physical exercise, etc.). It circulates in the blood either in a free form or bound to proteins such as α 2 macroglobulin and hGHBP (human growth hormone binding protein). Human GH triggers the hepatic synthesis of insulin-like growth factor (IGF1), which is responsible for the growth of long bones (indirect effect). The hormone also has metabolic effects, such as protein synthesis, nitrogen retention, the increase of plasmatic fatty acid levels and of hyperglycemic properties (stimulation of neoglucogenesis and hepatic glycogenolysis).

It must be emphasized that hGH-N gene transcription gives rise to 2 proteins:
- GH 22 kD, which has a biological activity;
- GH 20 kD, which has only a very weak biological activity (mainly a hyperglycemic effect).

The proportion of GH 20 kD compared to 22 kD in plasma is around 16%.

3. PRINCIPLE OF THE ASSAY

HGH-US is a solid phase two-site immunoradiometric assay. Two monoclonal antibodies were prepared against sterically remote antigenic sites on the hGH molecule. The first is coated on the solid phase (coated tube), while the second, radiolabelled with iodine 125, is used as a tracer.

The hGH molecules present in the calibrators or the samples to be tested are sandwiched between the two antibodies. Following the formation of the coated antibody/antigen/iodinated antibody sandwich, the unbound tracer is easily removed by a washing step.

The radioactivity bound to the tube is proportional to the concentration of hGH present in the sample.

4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

<table>
<thead>
<tr>
<th>REAGENTS</th>
<th>SYMBOLS</th>
<th>QUANTITY</th>
<th>STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>COATED TUBES: ready for use. Anti-hGH monoclonal antibody coated on the bottom of the tube.</td>
<td>CT</td>
<td>2 packs of 50 tubes</td>
<td>2-8°C until the expiry date. Tubes removed from their packs must be stored in the bag supplied with the kit.</td>
</tr>
<tr>
<td>ANTI-hGH 125I: ready for use. anti-hGH monoclonal antibody, buffer (Potassium Dihydrogen Phosphate, di-sodium hydrogen phosphate dihydrate, sodium caseinate, water), bovine albumin, sodium azide, non-immunized mouse immunoglobulins. ≤ 308 kBq (≤ 8.3 µCi).</td>
<td>TRACER</td>
<td>1 32 mL vial</td>
<td>2-8°C until the expiry date.</td>
</tr>
<tr>
<td>CALIBRATOR 0: lyophilized. Calf serum &amp; EDTA. Reconstitute with 2 mL of distilled water.</td>
<td>CAL</td>
<td>1 2 mL vial</td>
<td>2-8°C until the expiry date. After reconstitution, store 2 days at 2-8°C or 3 weeks at -20°C.</td>
</tr>
<tr>
<td>CALIBRATORS: lyophilized. Highly purified human hGH, calf serum &amp; EDTA. hGH concentrations: 0.3 - 3 - 15 - 37.5 - 75 µlU/ml.* Reconstitute with 0.5 mL of distilled water.</td>
<td>CAL</td>
<td>5 0.5 mL vials</td>
<td>2-8°C until the expiry date. After reconstitution, store 2 days at 2-8°C or 3 weeks at -20°C.</td>
</tr>
<tr>
<td>CONTROL: lyophilized. Highly purified human hGH, calf serum. 10.5 µlU/mL.* Reconstitute with 0.5 mL of distilled water.</td>
<td>CAL</td>
<td>1 0.5 mL vial</td>
<td>2-8°C until the expiry date. After reconstitution, store 2 days at 2-8°C or 3 weeks at -20°C.</td>
</tr>
<tr>
<td>TWEEN 20: concentrated solution. Dilute 9 mL of Tween 20 in 3 Liters of distilled water. Shake gently.</td>
<td>TWEEN 20</td>
<td>1 10 mL vial</td>
<td>2-8°C until the expiry date. After dilution, store in a capped container for 15 days maximum.</td>
</tr>
<tr>
<td>PLASTIC BAG</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

(*) The values shown above are only target values: the true value of each calibrator or control is shown on its label.
5. WARNING AND PRECAUTIONS

Safety measures
Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However, as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

All animal products are derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

It is highly recommended to consider the following recommendations when using in vitro assays:
- Do not pipette by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
- Avoid splashing.
- Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

Basic radioprotection rules
This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

The enforcement of the basic rules for handling radioactive products ensures adequate security.

A summary of these is given below:
- Radioactive products must be stored in their original containers in a suitable area.
- A record of the reception and storage of radioactive products must be kept up to date.
- Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
- Do not eat, drink, smoke or apply cosmetics in a controlled zone.
- Do not mouth-pipette radioactive solutions.
- Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
- Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
- Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
- All radioactive waste disposal must be carried out according to the regulations in force.

HGH-US kit is intended for in vitro diagnostic use.
HGH-US kit is intended for health professional only.
HGH-US kit is not intended to be used with automated systems

6. DILUTION AND RECONSTITUTION

Dilutions
Samples: Should elevated hGH levels be suspected, the dilution is performed with the calibrator 0 found in the kit. It is recommended that disposable plastic tubes be used when carrying out dilutions.
TWEEN 20: Dilute 9 mL of Tween 20 in 3 Liters of distilled water. Shake gently

Reconstitutions
Calibrators and controls: Reconstitute the controls with 0.5 mL distilled water. Reconstitute the calibrator 0 with 2mL of distilled water and the other calibrators with 0.5mL of distilled water.

7. STORAGE INSTRUCTIONS

The kit is shipped at room temperature and should be stored at 2°-8°C. Keep away from heat our direct sun light. The storage and stability period of each reagent (inclusive of reconstituted reagents) are indicated in section 4 of the instructions for use leaflet.

8. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum. If the assay is performed within 24 hours, the samples should be kept at 2-8°C. Otherwise, they should be divided into aliquots and stored deep frozen (-20°C).

9. ASSAY PROCEDURE

Material required but not provided
The following material is required but not provided in the kit (refer to section 4 for the list of reagents provided in the kit):
- Precision micropipettes or similar with disposable tips, capable of dispensing 50 µL, 300 µL, 500 µL and 2 mL. Their calibration should be checked regularly.
- Distilled water.
- Disposable plastic tubes.
- Circular horizontal shaker.
- Gamma scintillation counter calibrated for 125 iodine measurement.
Handling precautions
- Before starting the assay, read completely and carefully the instructions for use. Use the version of the package insert provided with the kit. Be sure that everything is understood prior starting.
- Do not use kit components beyond their expiry date.
- Do not mix reagents from different batches.
- Avoid any microbiotic contamination of the reagents or of the water used for washing.
- Fully respect the incubation conditions and the washing instructions indicated.
- All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use.
- Reconstitution and dispensing of the reagents into the tubes are also carried out at room temperature (18-25°C).

The assay requires the following groups of tubes:
- Calibrator 0 group for the determination of non-specific binding,
- Calibrator groups to establish the calibrator curve,
- Control group for the control,
- Sx groups for the samples to be assayed.

It is recommended to perform the assay in triplicate for calibrator groups, in duplicate for samples.

Protocol
Respect the order in which reagents are to be added:
1. Dispense 50 µL of calibrators, control or samples into the corresponding groups of tubes.
2. Add 300 µL of monoclonal anti-hGH tracer to each tube.
3. Incubate 2 hours ± 5 minutes at room temperature (18-25°C) under shaking (400 rpm).
4. Wash the coated tubes as follows:
5. Aspirate the content of the tubes as completely as possible.
6. Add 3.0 mL of washing solution (diluted tween 20) to each tube, wait 5 minutes before emptying them again.
7. Repeat the process one more.
8. Aspirate the contents of the tubes as completely as possible. There must be no residual volume in the coated tubes after washing.

To obtain reliable and reproducible results, the different washing steps have to be correctly performed. As much as possible of the incubation and washing solutions must be removed. If the washing is carried out manually, the tip of the aspirating device must be placed right at the bottom of the tube.

9. Measure the radioactivity bound to the coated tubes with a gamma scintillation counter.

Assay flowchart

<table>
<thead>
<tr>
<th>Groups of Tubes</th>
<th>Quantity (in µL) of Calibrators to add in the tubes</th>
<th>Quantity (in µL) of Sample to add in the tubes</th>
<th>Quantity (in µL) of (125^i) anti-hGH to add in the tubes</th>
<th>3 to 8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrators</td>
<td>50</td>
<td>-</td>
<td>300</td>
<td>Incubate for 2 hours ± 5mn under shaking at 18-25°C</td>
<td>Count</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>50</td>
<td>300</td>
<td>Wash 2 times with the washing solution</td>
<td></td>
</tr>
<tr>
<td>Samples (sx)</td>
<td>-</td>
<td>50</td>
<td>300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calibration
The calibrators are calibrated against the 2\textsuperscript{nd} international standard WHO IS 98/574 (Somatropin – NIBSC version dated 02 November 2000) \(1\text{ng} = 3 \mu\text{IU}\).
The calibrators range goes from 0 ng/mL to 75,00 µIU/mL.
The limit of detection of the assay was assessed at 0.03 µIU/mL.

Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended. Test results are valid only if the test has been performed following the instructions. Moreover, the user must strictly adhere to the rules of GLP or other applicable federal, state and local standards and/or law. All calibrators and controls must be found within the acceptable ranges as stated on the quality control certificate. If the criteria are not met, the run is not valid and should be repeated. It is recommended to take part to appropriate quality control trials.

In order to establish the calibration curve, the following procedure must be followed:
- For each group of tubes, calculate the mean counts after subtracting the background.
- Draw up the calibrator curve by plotting the calibrator's cpm against their concentrations.
- Use the "spline" mathematical function to establish the calibration curve.
Typical calibrator curve (example only): this data must under no circumstances be substituted for results obtained in the laboratory.

<table>
<thead>
<tr>
<th>Tube groups</th>
<th>Mean cpm</th>
<th>Concentration (µIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator 0</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Calibrator 1</td>
<td>373</td>
<td>0.21</td>
</tr>
<tr>
<td>Calibrator 2</td>
<td>2957</td>
<td>2.3</td>
</tr>
<tr>
<td>Calibrator 3</td>
<td>14213</td>
<td>12.2</td>
</tr>
<tr>
<td>Calibrator 4</td>
<td>31811</td>
<td>33.3</td>
</tr>
<tr>
<td>Calibrator 5</td>
<td>50108</td>
<td>65.6</td>
</tr>
<tr>
<td>Control</td>
<td>10622</td>
<td>9.9</td>
</tr>
<tr>
<td>A</td>
<td>5310</td>
<td>4.4</td>
</tr>
<tr>
<td>B</td>
<td>20321</td>
<td>19.3</td>
</tr>
<tr>
<td>C</td>
<td>35243</td>
<td>38.3</td>
</tr>
</tbody>
</table>

Results:
The hGH concentrations of the samples are determined by reading, on the x-axis, the sample values directly from the curve. In case of diluted samples, the values have to be multiplied with the corresponding the dilution factor.

10. LIMITATION OF THE METHOD

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results. Do not extrapolate sample values beyond the last calibrator. Samples showing a concentration above the highest calibrator should be diluted and re-assayed.

11. EXPECTED VALUES

The values given below are only indicative and it is recommended that each laboratory establish its own normal range. These values have been obtained from presumed healthy adult subjects from both sexes (n = 109). All values are in the 0 – 28.5 µIU/mL range and 93% are below 15 µIU/mL.

12. SPECIFIC PERFORMANCES

Precision
This has been assessed either using 2 samples with different concentrations 30 times in the same series of assay or with 4 samples in triplicate in 24 different series.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean (µIU/mL)</th>
<th>Within-run (C.V.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.1</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Recovery
Known quantities of hGH were added to human sera. The recovery percentages of hGH in the samples ranged from 95 to 110%.

Dilution test
Samples with high level were diluted. Recovery percentages obtained were between 100 and 122%.

Specificity
The antibodies used in this assay do not present any cross reaction with the structurally similar substances, hPL and prolactin. Furthermore, cross reaction with the 20kD hGH is less than 5% for concentration up to 3 750 µIU/mL (22kD hGH proportional concentration above 24 000 µIU/mL).

Interferences
No interference with bilirubin, haemoglobin, and triglycerides, measured up to respective concentrations of equal to 250 mg/L, 10 g/L, and 20 g/L, has been observed.
The immunoassay is protected against any human anti-mouse antibody (HAMA) interference by the addition of a protector to the tracer (non-specific mouse immunoglobulins). However, we cannot guarantee that this protection is exhaustive.

Detection limit
The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95%. It has been assessed as being 0.03 µIU/mL.

13. BIBLIOGRAPHY


14. SYMBOLS

<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>Meaning of symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>CE mark - Compliance with European Regulation</td>
</tr>
<tr>
<td></td>
<td>Storage temperature limitation</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch number</td>
</tr>
<tr>
<td>⏰</td>
<td>Use by date</td>
</tr>
<tr>
<td>⏰</td>
<td>Read the instructions for use</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic device</td>
</tr>
<tr>
<td>Manufactured by</td>
<td></td>
</tr>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>Σ</td>
<td>Number of determinations (the kit contains sufficient quantities of reagents for 100 determination)</td>
</tr>
<tr>
<td>CT</td>
<td>Coated tubes</td>
</tr>
<tr>
<td>TRACER</td>
<td>Radioactive tracer</td>
</tr>
<tr>
<td>CAL</td>
<td>Calibrator</td>
</tr>
<tr>
<td>TWEEN 20</td>
<td>Wash solution</td>
</tr>
</tbody>
</table>

15. MANUFACTURER AND DISTRIBUTOR IDENTIFICATION & LAST REVISION


HGH-US kit is distributed in the US by ALPCO Diagnostic, Inc located 26-G Keewaydin Dr. Salem, NH 03079, USA.

Last revision of the instructions for use: version14 from July 2016.