Human Growth Hormone (HGH) ELISA

For the quantitative determination of Growth Hormone (GH) in human serum.

For in vitro diagnostic use in the United States. For Research Use Only outside of the United States.

Catalog Number: 11-HGHHU-E01

Size: 96 Wells

Version: USA 10.0 – ALPCO 1.0

26-G Keewaydin Drive, Salem, NH 03079 | P: (800) 592-5726 | F: (603) 898-6854 | ts@alpco.com | www.alpco.com

INTENDED USE

For the quantitative determination of Growth Hormone (GH) in human serum.

For *in vitro* diagnostic use in the United States. For Research Use Only outside of the United States. Intended to be used manually but may be adaptable to open automated analyzers. The user is responsible for validating the performance of this kit with any automated analyzers.

LIMITATIONS RELATED TO INTENDED PURPOSE & USE

1. This test is not intended to be used for screening purposes.
2. This test is not intended for home testing or self-testing.
3. The kit is calibrated for the determination of GH in human serum. The kit is not calibrated for the determination of GH in other samples of human or animal origin.
4. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis and for therapeutic decisions.
5. Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as drugs and the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products have the potential of causing interferences.
6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received a preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

SUPPLEMENTAL INFORMATION

Human growth hormone (GH) is a polypeptide of 191 amino acids secreted by the somatroph cells of the anterior pituitary. Growth hormone is principally a regulator of body growth and its metabolic effects are primarily anabolic. Some of its effects include promotion of protein conservation through its involvement in a wide range of protein synthesis mechanisms, enhancement of glucose transport and facilitation of glycogen storage. In addition, it induces the release of somatomedins (insulin-like growth factors), which further mediate the cascade of growth promoting actions.

Measurement of GH is primarily of interest in the diagnosis and treatment of various forms of decreased secretion of GH. Hyposecretion of GH in children results in growth retardation and hypersecretion leads to gigantism in children and acromegaly in adults.

The secretion of GH varies throughout the day under the influence of intricate neurogenic, metabolic and hormonal control. Due to the pulsatile nature of GH release, it is often inaccurate to define a reference range and status based on single serum measurements. To diagnose disorders of GH secretion more reliably, dynamic tests are used in which serum GH levels are measured over a period following suppression or stimulation of GH secretion.

PRINCIPLE OF THE TEST

The HGH ELISA is a one-step capture or ‘sandwich’ type immunoassay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for GH is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of GH is conjugated to horse radish peroxidase (HRP conjugate).

In the first incubation step, GH present in the samples, calibrators and controls is simultaneously bound by the immobilized antibody and the HRP conjugate antibody, thus forming a sandwich complex. Excess and unbound materials are removed by a washing step.

Next, the TMB substrate (enzyme substrate) is added and reacts with HRP to form a blue colored product that is directly proportional to the amount of GH present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the color from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of GH in samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory *in vitro* use only.
2. Practice good laboratory practices when handling kit reagents and samples. This includes:
    1. Do not pipette by mouth.
    2. Do not smoke, drink, or eat in areas where samples or reagents are handled.
    3. Wear protective clothing and disposable gloves.
    4. Wash hands thoroughly after performing the test.
    5. Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiration date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and samples must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of samples.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will encounter any metal parts.
14. The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.

17. Samples values above the measuring range of the kit may be reported as > 50 ng/mL. If further dilution and retesting is required, only calibrator A may be used to dilute serum samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent the contamination of reagents, do not pour reagents back into the original containers.
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for SINGLE USE only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood samples. All human samples should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

The calibrators and controls provided with the kit contain material(s) of human origin that have been tested and found to be negative for the presence of HBsAg, HCV RNA and anti-HIV. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood sample, following good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SAMPLE COLLECTION, STORAGE, AND PRE-TREATMENT

Sample Collection and Storage

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labelled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum into a new storage tube or container. Serum samples may be stored at 2-8°C for up to 24 hours or at -10°C or lower if the analyses are to be done later.

Consider all human samples as possible biohazardous materials and take appropriate precautions when handling.

Sample Pre-Treatment

Specimen pre-treatment is not required.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 25 µL
2. Calibrated multi-channel pipettes to dispense 50 µL, 100 µL, and 300 µL (if washing manually)
3. Automatic microplate washer (recommended)
4. Microplate shaker:
    - Orbital shaker (3 mm diameter) set to 600 rpm or
    - Reciprocating shaker (1.5” stroke length) set to 180 oscillations/minute
5. Disposable pipette tips
6. Distilled or deionized water
7. Calibrated absorbance microplate reader with a 450 nm filter and upper OD limit of 3.0 or greater
8. Timer

REAGENTS PROVIDED

1. Microplate – Ready to Use

Contents: One anti-GH monoclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.

Storage: 2–8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

2. HRP Conjugate Concentrate – Concentrated; Requires Preparation

Contents: One bottle containing anti-GH monoclonal antibody-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 0.2 mL/bottle

Storage: 2–8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

Preparation of HRP Conjugate Working Solution: x101

Dilute 1:101 Before Use

Dilute 1:101 in assay buffer before use (e.g., 20 µL of conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 120 µL of conjugate concentrate in 12 mL of assay buffer. Discard any leftovers.

3. Calibrator A – F – Ready to Use

Contents: Six bottles of calibrator containing specified GH concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of GH. Calibrated against World Health Organization (WHO) 1st IS 80/505.

Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Concentrations: 0, 1, 5, 10, 25, 50 ng/mL

Volume: Calibrator A: 2.0 mL/bottle

Calibrator B-F: 0.5 mL/bottle

Storage: 2–8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

4. Control 1 – 2 – Ready to Use

Contents: Two bottles of control containing different GH concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of GH.

Refer to the QC certificate for the target values and acceptable ranges.

Volume: 0.5 mL/bottle

Storage: 2–8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

5. Assay Buffer – Ready to Use

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Volume: 15 mL/bottle

Storage: 2–8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

6. TMB Substrate - Ready to Use

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Volume: 16 mL/bottle

Storage: 2–8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

7. Stop Solution – Ready to Use

Contents: One bottle containing 1M sulfuric acid.

Volume: 6 mL/bottle

Storage: 2–8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

Safety:  **Warning!** Refer to product SDS.

8. Wash Buffer Concentrate – Concentrated; Requires Preparation

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 mL/bottle

Storage: 2–8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

Following Preparation: The 1x working wash buffer is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the

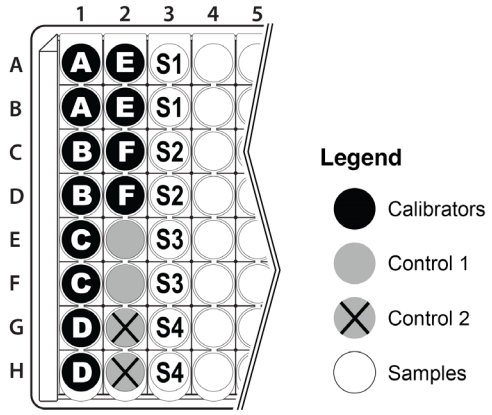
wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.

Preparation of 1x working Wash Buffer: X10

Dilute 1:10 Before Use

Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

Sample Pre-Treatment: None

All kit components, controls, and samples must reach room temperature prior to use. Calibrators, controls, and samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, mix gently by inversion.
2. Prepare the 1x Working HRP Conjugate and 1x Working Wash Buffer (See Reagents Provided section; HRP Conjugate Concentrate and Wash Buffer Concentrate).
3. Plan the microplate wells to be used for calibrators, controls, and samples. See Recommended Assay Layout. Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
4. Pipette 25 µL of each calibrator, control, and sample into assigned wells.
5. Pipette 100 µL of the HRP Conjugate Working Solution into each well (the use of a multi-channel pipette is recommended).
6. Incubate the microplate on a microplate shaker** for 60 minutes at room temperature.
7. Wash the microplate wells with an automatic microplate washer (preferred) or manually as stated below.
   Automatic: Using an automatic microplate washer, perform a 3-cycle wash using 300 µL/well of 1x Working Wash Buffer (3 x 300 µL). One cycle consists of aspirating all wells then filling each well with 300 µL of 1x Working Wash Buffer. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

Manually: For manual washing, perform a 3-cycle wash using 300 µL/well of 1x Working Wash Buffer (3 x 300 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 300 µL of 1x Working Wash Buffer into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.

8. Pipette 100 µL of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
9. Incubate the microplate on a microplate shaker** for 10-15 minutes at room temperature.
10. Pipette 50 µL of Stop Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
11. Measure the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stop Solution.

** See Reagents and Equipment Needed but Not Provided for microplate shaker options.

CALCULATIONS

1. Calculate the mean optical density for each calibrator, control, and sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 50 ng/mL and needs to be diluted and retested, then dilute with calibrator A not more than 1:10. The result obtained must be multiplied by the dilution factor.

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
2. The results of any external controls that were used meet the acceptable ranges.

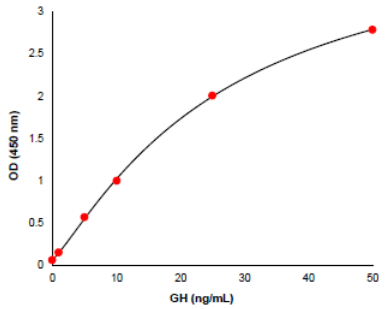
TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (ng/mL)
A	0.073	3	0
B	0.159	6	1
C	0.577	21	5
D	1.006	36	10
E	2.015	72	25
F	2.791	100	50
Unknown	0.555	-	5.0

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



PERFORMANCE CHARACTERISTICS

Sensitivity

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the HGH ELISA kit is 0.2 ng/mL.

Specificity (Cross-reactivity)

The specificity of the HGH ELISA kit was determined by measuring the apparent HGH value of calibrator A spiked with various levels of prolactin.

Substance	Concentration Range (ng/mL)	Apparent HGH Value (ng/mL)
Prolactin Calibrated against WHO 3rd IS 84/500	50 100 500 1000	Not Detected Not Detected Not Detected Not Detected

PRECISION

Intra-Assay Precision

Three serum samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below

Sample	Mean	SD	CV %
1	1.46	0.09	5.8
2	12.33	0.68	5.5
3	41.87	0.97	2.3

Inter-Assay Precision

Three serum samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below

Sample	Mean	SD	CV %
1	2.95	0.27	9.0
2	19.29	0.86	4.4
3	36.06	1.72	4.7

LINEARITY

Three serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1	6.44	-	-
1:2	3.12	3.22	96.9
1:5	1.15	1.29	89.1
1:10	0.59	0.64	92.2
2	16.60	-	-
1:2	7.97	8.30	96.0
1:5	2.82	3.32	84.9
1:10	1.59	1.66	95.8
3	33.00	-	-
1:2	16	16.5	97.0
1:5	6.4	6.6	97.0
1:10	3.3	3.3	100.0

RECOVERY

Spiked samples were prepared by adding defined amounts of GH to three serum samples. The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	ND	-	-
+ 1.0	0.96	1.0	96.0
+ 5.0	5.6	5.0	112.0
+ 50	49	50	98.0
2 Unspiked	0.7	-	-
+ 1.0	1.5	1.7	88.2
+ 5.0	6.6	5.7	115.8
+ 50	53	50.7	104.5
3 Unspiked	1.0	-	-
+ 1.0	1.7	2.0	85.0

+ 5.0	6.8	6.0	113.3
+ 50	48.8	51	95.7

REFERENCE RANGES

Each laboratory should collect data and establish their own range of expected normal values.

Group	N	95% Confidence Range (ng/mL)
Males	120	ND – 3.7
Females:		
Premenopausal	120	ND – 8.71
Postmenopausal	120	ND – 3.09

Product Complaints

In the case of product complaints, the user shall submit in writing to ALPCO a description of the complaint and provide accompanying data and/or information.

Warranty

ALPCO guarantees that the product is free of defects and will perform within the product specifications when the product is used prior to the expiration date, according to the intended purpose and use, and according to the instructions for use provided with the product. Any deviations from the intended purpose and use, instructions for use, modifications to kit components or use beyond the expiration date will invalidate any warranty claims.

Limitation of Liability

ALPCO liability in all circumstances whether in tort (including negligence) or at common law, and for any damage or loss, including but not limited to loss of profit and loss of sales, suffered whether direct, indirect, consequential, incidental or special is limited to the purchase price of the product(s) in question.

LITERATURE

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