



GH (Human) ELISA Kit

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96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

This GH (Human) ELISA Kit is to be used for the in vitro quantitative determination of human growth hormone (hGH) concentration in serum. This kit is intended FOR LABORATORY RESEARCH USE ONLY, and is not to be used in diagnostic or therapeutic procedures.

Background

Human growth hormone (GH) is a 22kDa monomeric protein produced and stored in somatotrophs in the anterior pituitary gland. GH is released from the pituitary into the bloodstream in a pulsatile manner under the regulatory control of hypothalamic somatostatin (SS) and GH-releasing factor (GHRF) [1]. The timing and frequency of GH release appears to be regulated by somatostatin, while the amplitude of GH release is determined by GHRF. A minor fraction (~10%) of GH in circulation exists in a smaller 20 kDa form [2]. GH has profound effects on tissue growth and metabolism, which is thought to be mediated through GH-dependent production of IGF-I and IGF-II, and their associated binding proteins. GH apparently stimulates IGF production after binding to specific cell surface receptors in the liver and, possibly, other tissues. Almost 50% of GH in circulation is bound to a high affinity GH binding protein (GHBP), which represents the extracellular domain of the GH receptor. Deficient GH secretion can occur in a number of clinical conditions [3]. However, evaluation of GH deficiency is complicated by the episodic nature of GH secretion and low circulating levels. A variety of physiologic and pharmacologic stimuli have been used to stimulate pituitary GH release during testing and failure to achieve a normal serum GH level in response to at least 2 GH stimulation or provocative tests is considered to be a diagnostic of GH deficiency [4]. The definition of a normal serum GH response is controversial, although published values generally range from 5 to 10 ng/mL. GH excess (or acromegaly) can be caused either by direct GH hypersecretion or GH excess secondary to GHRF hypersecretion.

Principle of the Assay

This hGH enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to hGH. Standards or samples are then added to the microtiter plate wells and incubated. After wash all wells, hGH if present, will bind to the antibody pre-coated on the wells. In order to quantitatively determine the amount of hGH present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody specific to hGH is added to each well to “sandwich” the hGH immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, a TMB (3,3', 5,5' Tetramethyl-benzidine) substrate solution is

added to each well. This enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain hGH and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.

In order to measure the concentration of hGH in the sample; this GH (Human) ELISA Kit includes a set of calibration standards (6 standards). The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density (O.D.) versus GH concentration (ng/mL). The concentration of hGH in the samples is then determined by comparing the O.D. of the samples to the standard curve.

General Information

Materials Supplied

List of component

Component	State	Amount
Microtiter Plate	One 96-well Microtiter Plate precoated with anti-human hGH monoclonal antibody.	96 wells
Conjugate	Anti-human hGH monoclonal antibody conjugated to horseradish peroxidase with preservative	12 mL
hGH Standard, 12 ng/mL	Lyophilized human hGH in a buffered protein base with preservative that will contain 12 ng/mL after reconstitution.	1 vial
hGH Standard, 6 ng/mL	Lyophilized human hGH in a buffered protein base with preservative that will contain 6 ng/mL after reconstitution.	1 vial
hGH Standard, 2 ng/mL	Lyophilized human hGH in a buffered protein base with preservative that will contain 2 ng/mL after reconstitution.	1 vial
hGH Standard, 0.8 ng/mL	Lyophilized human hGH in a buffered protein base with preservative that will contain 0.8 ng/mL after reconstitution.	1 vial
hGH Standard, 0.2 ng/mL	Lyophilized human hGH in a buffered protein base with preservative that will contain 0.2 ng/mL after reconstitution.	1 vial
hGH Standard, 0 ng/mL	Lyophilized buffered protein base with preservative that will contain 0 ng/mL after reconstitution.	1 vial
Sample Diluent	Animal serum in buffer with 0.05% proclin-300.	7 mL
Substrate A	Buffered solution with H ₂ O ₂	10 mL
Substrate B	Buffered solution with TMB.	10 mL
Stop Solution	2N Sulphuric Acid (H ₂ SO ₄). Caution: Caustic Material!	14 mL
Wash Buffer (20X)	20-fold concentrated solution of buffered surfactant	60 mL

Storage Instruction

All reagents provided are stored at 2-8°C. Refer to expiration date on the label.

Materials Required but Not Supplied

- ✓ Single or multi-channel precision pipettes with disposable tips: 10-100 µl and 50-200 µl for running the assay.
- ✓ Pipettes: 1 ml, 5 ml 10 ml, and 25 ml for reagent preparation.
- ✓ Multi-channel pipette reservoir or equivalent reagent container.
- ✓ Test tubes and racks.
- ✓ Polypropylene tubes or containers (25 ml).
- ✓ Incubator (37°C).
- ✓ Microtiter plate reader (450 nm \pm 2nm)
- ✓ Automatic microtiter plate washer or squirt bottle.
- ✓ Sodium hypochlorite solution, 5.25% (household liquid bleach).
- ✓ Deionized or distilled water.
- ✓ Plastic plate cover.
- ✓ Disposable gloves.
- ✓ Absorbent paper.

Precautions for Use

- Precautions
- ✓ Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
- ✓ Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
- ✓ Do not use kit components beyond their expiration date.
- ✓ Use only deionized or distilled water to dilute reagents.
- ✓ Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
- ✓ Use fresh disposable pipette tips for each transfer to avoid contamination.
- ✓ Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
- ✓ All samples should be disposed of in a manner that will inactivate human viruses.

Solid Wastes: Autoclave for 60 minutes at 121°C.

Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.

- ✓ Substrate Solution is easily contaminated. If bluish prior to use, do not use.
- ✓ The Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.

- Limitations of the Procedure
- ✓ The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated O.D. readings.

Assay Protocol

Reagent Preparation

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

- hGH standard: Reconstitute each hGH Standard vial with 0.6 mL of deionized or distilled water. Allow each solution to sit for at least 15 minutes with gentle agitation. The hGH standard stock solutions are stable at 4°C for 3 months. Avoid freeze-thaw cycles.
- Wash Buffer (1X): Dilute 1 volume of Wash Buffer (20X) with 19 volumes of distilled or deionized water. Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.
- Substrate Solution: Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
16 wells	1.5	1.5	3.0
32 wells	3.0	3.0	6.0
48 wells	4.0	4.0	8.0
64 wells	5.0	5.0	10.0
80 wells	6.0	6.0	12.0
96 wells	7.0	7.0	14.0

Sample Preparation

Serum: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted. This kit is for use with serum samples without additives only.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum samples to be used within 24-48 hours may be stored at 2-8°C otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

Assay Procedure

1. Prepare all hGH Standards before starting assay procedure (see Preparation Reagents). It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.
2. First, secure the desired number of coated wells in the holder, then add 50 μ L of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate. Then add 50 μ L of Sample Diluent to each well. COMPLETE MIXING IN THIS STEP IS IMPORTANT. Cover and incubate for 45 minutes at 37°C.
3. Wash the Microtiter Plate using one of the specified methods indicated below:
Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with wash buffer then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a total of FIVE washes. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.
Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.
Automated Washing: Aspirate all wells, then wash plates FIVE times using wash buffer. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μ L/well/wash (range: 350-400 μ L). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. It is recommended that the washer be set for soaking time of 10 seconds or shaking time of 5 seconds between washes.
4. Add 100 μ L of Conjugate into each well. Cover and incubate for 45 minutes at 37°C
5. Repeat wash procedure as described in Step 3.
6. Add 100 μ L of substrate into each well. Cover and incubate for 15 minutes at 37°C.
7. Add 100 μ L of Stop Solution to each well. Mix well.
8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

Data Analysis

Calculation of Results

This standard curve is used to determine the amount of follicle stimulating hormone (hGH) in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding hGH concentration (ng/mL) on the horizontal (X) axis.

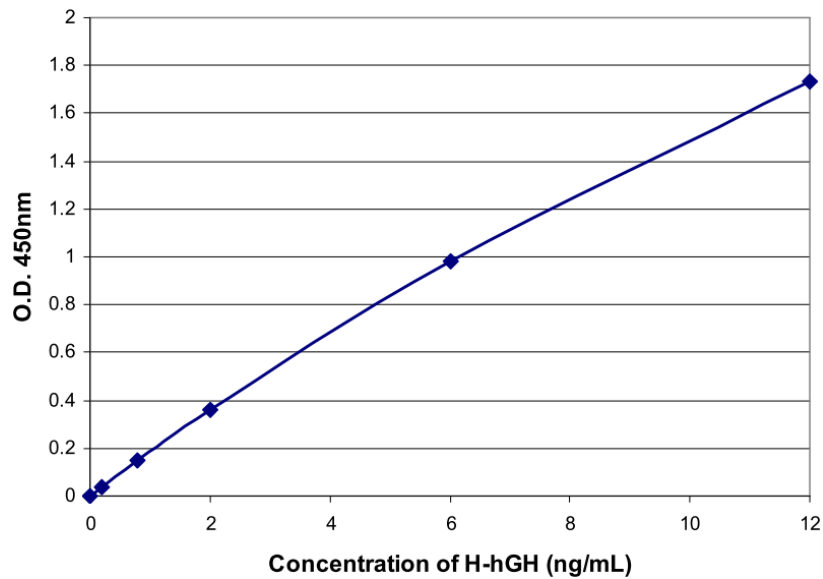
1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard (0 pg/ml) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of hGH in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding hGH concentration.

Results of a typical standard run of GH (Human) ELISA Kit are shown. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each users should obtain their own standard curve.

Example

Results of a typical standard run are shown below:

hGH (ng/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted
0	0.074, 0.070	0.072	0
0.2	0.114, 0.099	0.107	0.035
0.8	0.255, 0.221	0.238	0.149
2	0.442, 0.420	0.431	0.359
6	1.075, 1.030	1.052	0.980
12	1.807, 1.798	1.803	1.731



Performance Characteristics

- **Sensitivity**
The minimal detectable concentration of hGH by this assay is estimated to be 0.1 ng/mL.
- **Specificity**
This kit exhibits no detectable cross-reaction with LH, hCG, TSH, Prolactin, and FSH.
- **Calibration**
This immunoassay is calibrated against NIBSC/WHO, 98/574, hGH
- **Hook effect**
In this assay, no hook effect is observed.
- **Expected Normal Values**
Each laboratory must establish its own normal range. The results provided below are based on random selected outpatient clinical laboratory samples:

Sample	N	Range (ng/mL)
Serum	50	< 7

Resources

References

1. Iranmesh A, et al. J Clin Endocrinol Metab 73:1081-1088, 1991.
2. Smal J, et al. Biochem Biophys Res Comm 134:159-165, 1986.
3. Frasier SD. Endocrin Rev 4:155-170, 1983.
4. Ad Hoc Committee on Growth Hormone Usage, et al. Pediatrics 72:891894, 1983.

Plate Layout

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