Human Growth Hormone ELISA Kit

Human Growth Hormone ELISA Kit is an Enzyme Immunoassay kit for the quantification of Human Growth Hormone in serum.

Catalog number: ARG82808

Package: 96 wells

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INTRODUCTION

Growth hormone (GH) or somatotropin, also known as human growth hormone (hGH or HGH) in its human form, is a peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals. It is thus important in human development. GH also stimulates production of IGF-1 and increases the concentration of glucose and free fatty acids. It is a type of mitogen which is specific only to the receptors on certain types of cells. GH is a 191-amino acid, single-chain polypeptide that is synthesized, stored and secreted by somatotropic cells within the lateral wings of the anterior pituitary gland.

A recombinant form of hGH called somatropin (INN) is used as a prescription drug to treat children's growth disorders and adult growth hormone deficiency. In the United States, it is only available legally from pharmacies by prescription from a licensed health care provider. In recent years in the United States, some health care providers are prescribing growth hormone in the elderly to increase vitality. While legal, the efficacy and safety of this use for HGH has not been tested in a clinical trial. Many of the functions of hGH remain unknown.

In its role as an anabolic agent, HGH has been used by competitors in sports since at least 1982, and has been banned by the IOC and NCAA. Traditional urine analysis does not detect doping with HGH, so the ban was not enforced until the early 2000s, when blood tests that could distinguish between natural and artificial HGH were starting to be developed. Blood tests conducted by WADA at the 2004 Olympic Games in Athens, Greece targeted primarily HGH.

Use of the drug for performance enhancement is not currently approved by the FDA. [Provide by Wikipedia: Growth hormone]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for hGH has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any hGH present is bound by the immobilized antibody. After washing away any unbound substances, added antibody-conjugate specific for hGH to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of hGH bound in the initial step. The color development is stopped by the addition of stop solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of hGH in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	96 wells	4°C
Standards (lyophilized) 50 ng/mL	1 vial	4°C
Standards (lyophilized) 25 ng/mL	1 vial	4°C
Standards (lyophilized) 12 ng/mL	1 vial	4°C
Standards (lyophilized) 6 ng/mL	1 vial	4°C
Standards (lyophilized) 2 ng/mL	1 vial	4°C
Standards (lyophilized) 0 ng/mL	1 vial	4°C
Antibody Conjugate	12 mL (ready to use)	4°C
20X Wash Buffer	60 mL	4°C
Diluent Buffer	7 mL	4°C
Substrate A	10 mL	4°C
Substrate B	10 mL	4°C (protect from light)
Stop Solution	14 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm
- Incubator (37°C)
- Deionized or distilled water
- Sodium hypochlorite solution, 5.25% (household liquid bleach)
- Pipettes and pipette tips
- Plastic plate cover
- Multichannel micropipette reservoir
- Microtiter plate shaker (recommended)
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- 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.
- Store the kit at 2-8°C at all times.
- Return any unused microplate strips to the plate pouch with desiccant.
- Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
- The Substrate B contains 20% acetone, keep reagent away from sources of heat or flame.

- All samples should be disposed of in a manner that will inactivate human viruses.

  Solid Waste: Autoclave 60 minutes at 121°C.

  Liquid Waste: 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 viruses before disposal.
- The reagent preparation method might be different from lot to lot, so please check the lot and follow the instructions given in this manual.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 60 minutes. Centrifuge at 1,000 x g for 10 minutes at 4°C. This kit is for use with serum samples without additives only.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Samples containing sodium azide should not be used in the assay.
3. 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 repeated freeze-thaw cycles.
4. DO NOT USE HEAT-TREATED SPECIMENS.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 30 mL of 20X Wash Buffer into 570 mL of distilled water to a final volume of 600 mL). Wash Buffer is stable for 1 month at 2-8°C.
- Working 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.

Used Wells	Substrate A (mL)	Substrate B (mL)	Working 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

- Standards: Reconstitute each 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 Standard stock solutions are stable at 4°C for 3 months. Avoid freeze-thaw cycles.

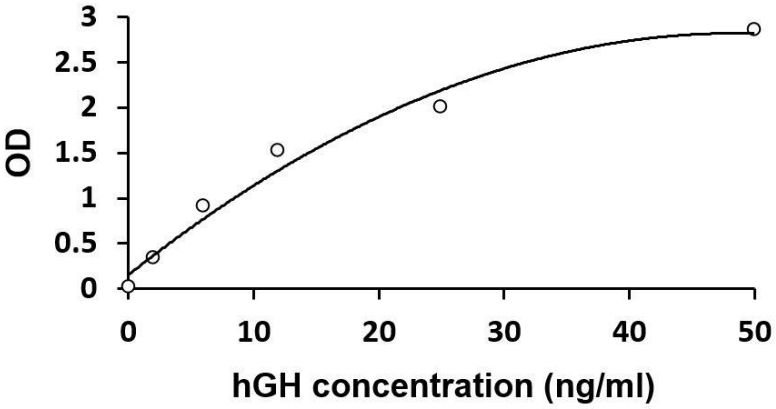
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **50 µL** of **samples** and **each Standards** to the **Antibody Coated Microplate**. Then add **50 µL** of **Diluent Buffer** to each well. **COMPLETE MIXING IN THIS STEP IS IMPORTANT**. Incubate for **30 minutes** at **37°C.**
2. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with **1X Wash Buffer (350 µL)** using a squirt bottle, manifold dispenser. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. **Note:** Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.
3. Add **100 µL** of the **Antibody Conjugate** per well. Then cover the plate and incubate for **30 minutes** at **37°C.**
4. Aspirate and wash plate as in step 3.
5. Add **100 µL** of the **Working Substrate Solution** per well. Then cover the plate and incubate for **15 minutes** at **37°C.**
6. Immediately Add **100 µL** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at **450 nm** within **30 minutes**.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human Growth Hormone ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the Standard (0 ng/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

0.5 ng/mL

Specificity

This kit exhibits no detectable cross-reaction with LH, hCG, TSH, Prolactin, and FSH.