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 For Order and Inquiries, please contact:  
 Immuno-Biological Laboratories, Inc. (IBL-America)  
 8201 Central Ave NE, Suite P  
 Minneapolis, MN 55432  
 Tel (763) 780-2955, Fax (763) 780-2988,  
[www.ibl-america.com](http://www.ibl-america.com)



# Human Growth Hormone (hGH) ELISA

Catalog No. IB19101N (96 Tests)

**INTENDED USE**

The hGH ELISA kit is used for the measurement of hGH in human serum or plasma. For research use only – Not for use in Diagnostic Procedure.

**SUMMARY AND EXPLANATION**

Human Growth Hormone (hGH) is a polypeptide chain, composed of 191 amino acids and with a molecular weight of 21,500. It is released by the anterior pituitary of both men and women. The secretion is stimulated 3-4 hours after a meal, about 1 hour after the beginning of sleep and after physical exercise.

Hyposecretion of hGH becomes apparent in infants a few months after birth and may result in dwarfism. In the opposite case, hypersecretion of hGH results in gigantism and may be due to hypophyseal tumors. In adults, when epiphyses are closed, hypersecretion of hGH provokes an increase in volume of soft tissues (hands, feet, lips) and a proliferation of bones (acromegaly syndrome) and a limited tolerance of glucose.

hGH has profound effects on tissue growth and metabolism, which is thought to be mediated through GH-dependent production of Insulin-like Growth Factor (IGF) I and IGF-II, and their associated binding proteins. hGH apparently stimulates IGF production after binding to specific cell surface receptors in the liver. The major target tissues affected by the IGF-1 in combination with the hGH signal are muscle, cartilage, bone, liver, kidney, nerves, skin and lungs. Evaluation of hGH deficiency is complicated by the episodic nature of hGH secretion and low circulating levels. A variety of physiologic and pharmacologic stimuli have been used to stimulate pituitary hGH release during testing and failure to achieve a normal serum hGH level in response to at least 2 hGH stimulation or provocative tests is considered to be a diagnostic of hGH deficiency. The definition of a normal serum hGH response is controversial, although published values generally range from 5 to 10 ng/ml.

**PRINCIPLE OF THE TEST**

The hGH ELISA is based on a solid phase sandwich ELISA method. The samples and conjugate reagent (anti-hGH biotin & HRP) are added to the wells coated with Streptavidin. hGH in the serum binds to the matched pair Abs, forming a sandwich complex and simultaneously the complex is being immobilized on the plate through streptavidin-biotin interactions. Unbound protein and HRP conjugate are washed off, through a washing step. Upon addition of the substrate, the intensity of color is proportional to the concentration of hGH in the samples. A standard curve is prepared relating color intensity to the concentration of the hGH.

MATERIALS PROVIDED	96 Tests
1. Microwell coated with Streptavidin	12x8x1
2. hGH Standard: 6 vials (ready to use)	0.5ml
3. hGH Conjugate Reagent: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12ml
5. Stop Solution: 1 bottle (ready to use)	12ml
6. 20X Wash concentrate: 1 bottle	25ml

**MATERIALS NOT PROVIDED**

1. Distilled or deionized water
2. precision pipettes
3. Disposable pipette tips
4. Micortiter well reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

**STORAGE AND STABILITY**

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagent to heat, sun, or strong light.

**WARNINGS AND PRECAUTIONS**

1. Potential biohazardous materials:  
The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. This kit is designed for research use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

**SPECIMEN COLLECTION HANDLING**

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8° C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

**REAGENTS PREPARATION**

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26° C).

**ASSAY PROCEDURE**

Prior to assay, allow reagents to stand at room temperature.

Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 50 µl of hGH standards, control and sera into the appropriate wells.
3. Add 100 µl of hGH conjugate reagent to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (20-25° C).
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

**CALCULATION OF RESULTS**

The standard curve is constructed as follows:

1. Check hGH standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the hGH standards (vertical axis) versus the hGH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
4. Value above the highest point of the standard are retested after diluting with "0" standard.

**Example of a Standard Curve**

Standard	OD (450 nm)
Standard 1 (0 µIU/mL)	0.006
Standard 2 (2 µIU/mL)	0.123
Standard 3 (10 µIU/mL)	0.465
Standard 4 (25 µIU/mL)	1.004
Standard 5 (50 µIU/mL)	1.503
Standard 6 (150 µIU/mL)	2.328

**RESULTS**

Results are expressed in µIU/mL. To convert to ng/mL, divide results by 3.7.

Example: 10 µIU/mL/3.7 = 2.7 ng/mL

**LIMITATIONS OF THE TEST**

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.