



Growth Hormone (human) ELISA Kit

rev 10/18

(Catalog # K7412-100, 100 assays; Store at 2-8°C)

I. Introduction:

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 hypophysic 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 (acromegalysyndrome) 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.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Human Growth Hormone

IV. Sample Type:

- Serum or plasma

V. Kit Contents:

Components	K7412-100	Part No.
Microplate coated with hGH MAb, 96 wells	12 stripsx8 wells	K7412-100-1
hGH Standard: (0.5 ml) (ready to use)	6 vials	K7412-100-2
hGH Enzyme Conjugate (ready to use)	12 ml	K7412-100-3
Wash Concentrate (20X)	25 ml	K7412-100-4
TMB Substrate (ready to use)	12 ml	K7412-100-5
Stop Solution (ready to use)	12 ml	K7412-100-6

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Concentrate:** 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).

VIII. Warning & Precautions:

- 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.
- This test kit is USA FDA exempt product.
- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.
- 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.

IX. Sample Preparation and Storage:

Collect blood specimens and separate the serum immediately. 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. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

X. Assay Protocol:

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use. 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.

1. Place the desired number of coated strips into the holder
2. Pipet 50 µl of hGH standards, control and patient's sera onto 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.

XI. Calculation:

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: $10\mu\text{IU/mL}/3.7 = 2.7\text{ng/mL}$

XII. RELATED PRODUCTS:

Human CellExp™ HGH, human recombinant (6457)
Growth hormone, human recombinant (4769)
Chicken Growth hormone Antibody (6648)

Growth hormone, chicken recombinant (4771)
Growth hormone, murine recombinant (4770)
Growth hormone Antibody (5769)

FOR RESEARCH USE ONLY! Not to be used on humans.