LIMITATIONS OF THE TEST

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

REFERENCES

1. L´opez-Guajardo CC; Armstrong LS; Jordan L; Staten NR; Krivi GG; Martinez AO; Haro LS. Generation, characterization and utilization of anti-human growth hormone 1-43, (hGH1-43), monoclonal antibodies in an ELISA. J Immunol Methods 1998; 215(1-2): 179-85.
2. Potter MA; Hymus S; Stockley T; Chang PL. Suppression of immunological response against a transgene product delivered from microencapsulated cells. Hum Gene Ther 1988; 9(9): 1275-82.
3. Strasburger CJ; Wu Z; Pflaum CD; Dressend¨orfer RA. Immunofunctional assay of human growth hormone (hGH) in serum: a possible consensus for quantitative hGH measurement. J Clin Endocrinol Metab 1996; 81(7): 2613-20.
4. Tsushima T; Katoh Y; Miyachi Y; Chihara K; Teramoto A; Irie M; Hashimoto Y. Serum concentration of 20K human growth hormone (20K hGH) measured by a specific enzyme-linked immunosorbent assay. Study Group of 20K hGH. J Clin Endocrinol Metab, 1999; 84(1): 317-22.

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Calbiotech Inc.,

CALBIOTECH
A life science company

Human Growth Hormone (hGH) ELISA

Catalog No. HG048H (96 Tests)

INTENDED USE

For Research Use Only. Not for use in diagnostic procedures.

MATERIALS PROVIDED	96 Tests
1. Microwell coated with hGH MAb	12x8x1
2. hGH Standard: 6 vials (ready to use)	0.5ml
3. hGH Enzyme Conjugate: 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. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. 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.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. 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. Typically, 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 (20-25°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 patient's sera.
3. Add 100 µl of hGH enzyme conjugate to all wells.
4. Cover the plate and incubate for 30 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 10 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 ng/ml)	0.025
Standard 2 (2.5 ng/ml)	0.199
Standard 3 (5 ng/ml)	0.359
Standard 4 (10 ng/ml)	0.709
Standard 5 (20 ng/ml)	1.287
Standard 6 (40 ng/ml)	2.430