ab108643

Human Growth Hormone
Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Growth Hormone concentrations in serum

This product is for research use only and is not intended for diagnostic use.

Version 2 Last updated 1 February 2019
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1. Introduction

ab108643 Human Growth Hormone Human ELISA Kit is intended for the quantitative determination of the Human Growth Hormone (HGH) concentration in human serum.

Human Growth Hormone (HGH, somatotropin) is a polypeptide secreted by the anterior pituitary. It is 191 amino acids in length and has a molecular mass of approximately 22,000 daltons. Its metabolic effects are primarily anabolic. Human Growth Hormone promotes protein conservation and is engaged in a wide range of mechanisms for protein synthesis. It also enhances glucose transport and facilitates glycogen storage. Its cascade of growth-promoting action is mediated by another family of peptide hormones, the somatomedins. Human Growth Hormone measurement is primarily of interest in the diagnosis and treatment of various forms of abnormal growth hormone secretion. Disorders caused by hyposecretion include dwarfism and unattained growth potential, and hypersecretion is associated with gigantism and acromegaly.
2. Assay Summary

*ab108643 is based on the principle of a solid phase enzyme-linked immunosorbent assay.*

The assay system utilizes a sheep anti-Human Growth Hormone antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-Human Growth Hormone antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution.

The test sample is allowed to react simultaneously with the antibodies, resulting in Human Growth Hormone molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45-minute incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies.

A solution of tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of a blue color.

The color development is stopped with the addition of 1N hydrochloric acid (HCl) changing the color to yellow. The concentration of Human Growth Hormone is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.
3. Kit Contents

- Antibody-Coated Wells (1 plate, 96 wells); microtiter wells coated with Sheep Anti-Human Growth Hormone.
- Reference standard set, containing 0, 2.5, 5, 10, 25, and 50 ng/mL Human Growth Hormone. Ready to use.
- Enzyme Conjugate Reagent, 13 ml.
- TMB Reagent (11 ml) contains one-step TMB solution.
- Stop Solution (11 ml) contains diluted hydrochloric acid (1N HCl).

4. Storage and Handling

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.
5. Additional Materials Required

- Distilled or deionized water
- Precision pipettes: 50 μl, 100 μl and 1.0 ml
- Disposable pipette tips
- Microtiter well reader capable of reading absorbance at 450 nm, with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater.
- Vortex mixer, or equivalent
- Absorbent paper
- Graph paper

6. Preparation of Reagents

1. All reagents should be allowed to reach room temperature (18-25°C) before use.

7. Preparation and Collection of Specimen

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.
8. Assay Method

Assay Procedure:

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450nm with a microtiter well reader within 15 minutes.
9. Data Analysis

1. Calculate the mean absorbance value ($A_{450}$) for each set of reference standards, controls and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration Human Growth Hormone in ng/ml) from the standard curve.

A. Typical Data

Results of a typical standard run with absorbency readings at 450nm shown on the Y axis against Human Growth Hormone concentrations shown on the X axis.

**NOTE:** This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each
laboratory must provide its own data and standard curve in each experiment.

<table>
<thead>
<tr>
<th>HGH (ng/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.052</td>
</tr>
<tr>
<td>2.5</td>
<td>0.392</td>
</tr>
<tr>
<td>5</td>
<td>0.641</td>
</tr>
<tr>
<td>10</td>
<td>1.125</td>
</tr>
<tr>
<td>25</td>
<td>1.946</td>
</tr>
<tr>
<td>50</td>
<td>2.610</td>
</tr>
</tbody>
</table>
B. Sensitivity

The minimum detectable concentration of the Human Growth Hormone ELISA assay is 0.5 ng/ml.
10. Limitations

- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
- The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
- Each laboratory must establish its own normal ranges based on population. A normal range for human growth hormone levels is difficult to define because of the normal physiological fluctuations in Human Growth Hormone concentration. In most adult subjects at rest, after an overnight fast, the Human Growth Hormone level in serum is 7 ng/ml or less. Changes in Human Growth Hormone levels in response to various stimuli give a more accurate assessment of pituitary dysfunction. Confirmation of diagnosis requires provocative tests, either stimulation or suppression.
## 11. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td>---------</td>
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<td>---------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
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</tbody>
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For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).