1. hGH levels are measured over a period following suppression secretion more reliably, dynamic tests are used in which serum control. Due to the pulsatile nature of hGH release, it is often retardation and hypersecretion leads to gigantism in children hGH. Hyposecretion of hGH in children results in growth and treatment of various forms of decreased secretion of growth promoting actions.

2. Mouse Anti-hGH Antibody-Coated-Break-Apart Well Microplate — Ready To Use

3. Mouse Anti-HCG Antibody-Conjugate-Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation

4. Controls — Ready To Use

5. Wash Buffer Concentrate

6. Assay Buffer — Ready To Use

7. TMB Substrate — Ready To Use

8. Stopping Solution

9. Calibrators

10. CONTROL SPECIMENS

11. CHEMICAL HAZARDS

12. SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARD MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and also has been tested for the presence of anti-HIV and Human Immunodeficiency Virus (HIV) and found to be negative. No testing method however may guarantee complete assurance that HIV, Hepatitis B and Hepatitis B virus or any infectious agents are removed. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate test. If the analysis is to be delayed for a later date, consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no pretreatment is needed.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 50, 100 and 300 µL
2. Disposable pipette tips
3. Diluted or distilled water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS

1. TMB Substrate

2. Stopping Solution

3. Mouse Anti-hGH Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate

4. Controls

5. Wash Buffer Concentrate

6. Assay Buffer

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REAGENTS

1. TMB Substrate

2. Stopping Solution  

3. Mouse Anti-hGH Antibody-Coated-Break-Apart Well Microplate — Ready To Use

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

2. Mouse Anti-HCG Antibody-Conjugate-Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Volume: 200 µL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in assay buffer before use (eg. 20 µL of HRP in 2 ml of assay buffer). If the whole plate is to be used diluted 120 µL of HRP in 12 ml of assay buffer. Discard any that is left over.

3. nGH Calibrators — Ready To Use


4. Controls — Ready To Use

Contents: Two vials containing hGH in a serum-based buffer with a non-mercury preservative. Prepared by spiking serum with defined quantities of hGH. Refer to vial labels for the acceptable range.

Volume: 50 µL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen.

Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate — Requires Preparation

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 µL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in diethyl or deionized water before use. If the whole plate is to be used dilute 50 µL wash buffer concentrate in 450 mL of water.

6. Assay Buffer — Ready To Use

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Volume: 15 µL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

7. TMB Substrate — Ready To Use

Contents: One bottle containing tetramethylbenzidine and 3-amino-9-ethylcarbazole in a non-DMF and DMSO containing buffer.

Volume: 600 µL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

8. Stopping Solution — Ready To Use

Contents: One bottle containing 1M sulfuric acid.

Volume: 6 µL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator Concentration Volume/Vial

Calibrator A 0.5 ng/mL 2.0 µL

Calibrator B 1 ng/mL 0.5 µL

Calibrator C 5 ng/mL 0.5 µL

Calibrator D 10 ng/mL 0.5 µL

Calibrator E 25 ng/mL 0.5 µL

Calibrator F 50 ng/mL 0.5 µL

Storage: Refrigerate at 2–8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen.

Avoid multiple freezing and thawing cycles.

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 µL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in diethyl or deionized water before use. If the whole plate is to be used dilute 50 µL wash buffer concentrate in 450 mL of water.
**ASSAY PROCEDURE**

Specimen Pretreatment: None.

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the anti-hGH-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 25 μL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 μL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Wash the wells 3 times with 300 μL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 100 μL of TMB substrate into each well at room temperature (or until calibrator F attains dark blue colour for desired OD).
8. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD). Do not return to 4°C at any time. Allow to develop until yellow colour is visible.
9. Pipette 50 μL of stopping solution into each well at the same time intervals as in step 7.
10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

**INTER-ASSAY PRECISION**

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.95</td>
<td>0.27</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>19.29</td>
<td>0.86</td>
<td>4.4</td>
</tr>
<tr>
<td>3</td>
<td>36.06</td>
<td>1.72</td>
<td>4.7</td>
</tr>
</tbody>
</table>

**RECOVERY**

Spiked samples were prepared by adding defined amounts of hGH to three patient serum samples. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ND</td>
<td>0.158</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>0.158</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>0.158</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**HIGH DOSE HOOK EFFECT**

The Direct hGH ELISA kit did not experience any high dose hook effect.

**EXPECTED VALUES**

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

**REFERENCES**