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Human Growth Hormone ELISA Kit

Catalog No. **CKH344**

Quantity **1 x 96 tests**

Introduction

Growth hormone (GH) known also as Somatotropic hormone or Somatotropin is a hormone that stimulates growth and cell reproduction in humans and other animals. GH is a single chain polypeptide hormone of 191 amino acids mainly produced by the adenohypophysis (anterior pituitary) and encoded by the GH1 (growth hormone 1) gene. It is synthesized, stored, and secreted by the somatotroph cells within the lateral wings of the anterior pituitary gland. GH has a variety of functions in the body, the most noticeable of which is the increase of height throughout childhood, and there are several diseases which can be treated through the therapeutic use of GH.

The Human GH ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human GH in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human GH coated on a 96-well plate. Standards and samples are pipetted into the wells and GH present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human GH antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of GH bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents:

1. Insulin Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human Insulin.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution
3. Standards (Item C): 2 vials of recombinant human GH.
4. Assay Diluent A (Item D): 30 ml animal serum with 0.09% sodium azide preservative. For Standard/Sample (serum/plasma) diluent.
5. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Standard/Sample (cell culture medium/urine) diluent.
6. Detection Antibody GH (Item F): 2 vials of biotinylated anti-human GH (each vial is enough to assay half microplate).
7. HRP-Streptavidin Concentrate (Item G): 8 µl 10,000x concentrated HRP-conjugated streptavidin.
8. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution.
9. Stop Solution (Item I): 8 ml of 2 M sulfuric acid.

Storage:

May be stored for up to 6 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at –80°C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.
Note: the kit can be used within one year if the whole kit is stored at -20°C .
Avoid repeated freeze-thaw cycles.

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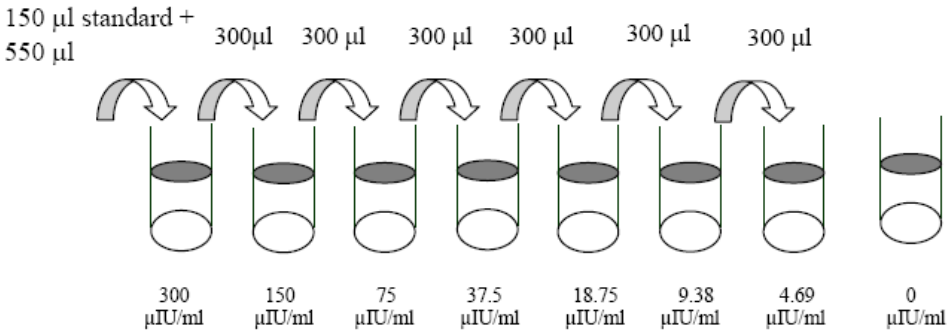
Additional Materials Required:

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Shaker
3. Precision pipettes to deliver 2 µl to 1 ml volumes.
4. Adjustable 1-25 ml pipettes for reagent preparation.
5. 100 ml and 1 liter graduated cylinders.
6. Absorbent paper.
7. Distilled or deionized water.
8. Log-log graph paper or computer and software for ELISA data analysis.
9. Tubes to prepare standard or sample dilutions.

Reagent Preparation:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) is used for dilution of serum/plasma samples, and 1x Assay Diluent B (Item E) can be used for dilution of culture supernatants/urine.
3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
4. Preparation of standard: **Briefly spin the vial of Item C** and then add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernates/urine) into Item C vial to prepare a 1,400 µIU/ml standard. **Dissolve the powder thoroughly by a gentle mix.** Add 150 µl Insulin standard (1,400 µIU/ml) from the vial of Item C, into a tube with 550 µl Assay Diluent A or 1x Assay Diluent B to prepare a 300 µIU/ml standard solution. Pipette 300 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the 300 µIU/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Gently vortex to mix. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 µIU/ml).



5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

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6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use HRP-Streptavidin concentrate should be diluted 25,000-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 2 µl of HRP-Streptavidin concentrate into a tube with 198 µl 1x Assay Diluent B to prepare a 100-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix through and then pipette 60 µl of prepared 100-fold diluted solution into a tube with 15 ml 1x Assay Diluent B to prepare a final 25,000 fold diluted HRP-Streptavidin solution.

Assay Procedure:

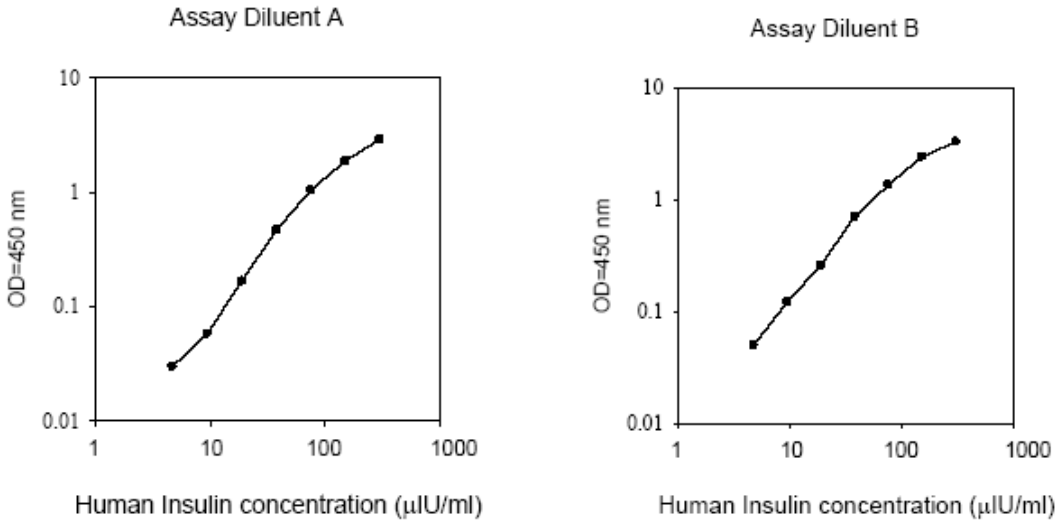
1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Assay Procedure Summary:

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well.
   Incubate 2.5 hours at room temperature or over night at 4°C.
3. Add 100 µl prepared biotin antibody to each well.
   Incubate 1 hour at room temperature.
4. Add 100 µl prepared Streptavidin solution.
   Incubate 45 minutes at room temperature
5. Add 100 µl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well.
   Read at 450 nm immediately.

Calculation of Results: Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay.



Sensitivity: The minimum detectable dose of Insulin is typically less than 4 µIU/ml.

Recovery: Recovery was determined by spiking various levels of human Insulin into human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	91.40	83-102
Plasma	99.03	73-128
Cell culture media	76.16	68-88

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Linearity:

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	100.2	109.5	86.69
	Range (%)	90-108	102-128	72-103
1:4	Average % of Expected	122.1	131.7	82.43
	Range (%)	112-135	121-140	68-90

Reproducibility: Intra-Assay: CV<10%
Inter-Assay: CV<12%

Specificity: Detect Insulin and Proinsulin.

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: human BDNF, BLC, ENA-78, IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIP-1α, MIP-1 β, MIP-1δ, PARC, PDGF, RANTES, SCF, TARC, TGF-β, TIMP-1, TNF-α, TNF-β, TPO, VEGF.

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Trouble-Shooting Guide:

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1.Too brief incubation times	1. Ensure sufficient incubation time; assay procedure step 2 change to over night
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using an a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the ELISA kit	1. Store your standard at<-20°C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	2. Stop solution	2. Stop solution should be added to each well before measure

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