

PATHOZYME[®] HUMAN GROWTH HORMONE Ref OD437

Enzyme Immunoassay EIA for the quantitative detection of hGH

in human serum.

Store at 2°C to 8°C. DO NOT FREEZE.

For in-vitro use only.

INTRODUCTION

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. hGH 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. hGH 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; hypersecretion is associated with gigantism and acromegaly.

Caution must be exercised in the clinical interpretation of growth hormone levels. These vary throughout the day, making it difficult to define a normal range or to judge an individual's status based on a single determination. Many factors are known to influence the rate of growth hormone secretion, including periods of sleep and wakefulness, exercise, stress, hypoglycemia, oestrogens, corticosteroids and L-dopa. Because of its similarity to prolactin and placental lactogen, earlier growth hormone immunoassays were often plagued with falsely high values in pregnant and lactating women. Because not all acromegalic individuals have elevated baseline levels of growth hormone, suppression tests based on glucose loading are of value in this context. In spite of the induced hyperglycemia, there is rarely a decrease from baseline levels in acromegaly.

Growth hormone-deficient individuals have fasting and resting levels similar to those found in normal individuals. Various challenge tests have therefore been devised to differentiate them. For example, with the onset of deep sleep or after 15 to 20 minutes of vigorous exercise, growth hormone levels normally rise.

Other tests for growth hormone responsiveness are based on the administration of L-dopa, arginine and insulin. Propanolol or oestrogen are sometimes given in conjunction with the primary stimulus to accentuate the response.

A small number of dwarfism cases have been documented in which both the basal level of hGH and the responses to challenge testing were normal. Such cases may involve tissue insensitivity to either growth hormone or the somatomedins, or immunoreactive but biologically inactive growth hormone.

The PATHOZYME hGH assay provides a rapid, sensitive and reliable test. There is no cross-reactivity with hCG, TSH, LH, and Prolactin.

INTENDED USE

PATHOZYME hGH is an Enzyme Immunoassay (EIA) for the quantitative determination of Human Growth Hormone (hGH) in human serum.

For professional use only.

PRINCIPLE OF THE TEST

Specific sheep anti-hGH antibodies are coated on to microtitre wells. Test sera are applied. Then monoclonal anti-hGH labelled with Horseradish Peroxidase enzyme (Conjugate) is added. If human hGH is present in the sample, it will combine with the antibody on the well and the enzyme Conjugate, resulting in the hGH molecules being sandwiched between the solid phase and the enzyme linked antibodies. After incubation at room temperature, the wells are washed with distilled water to remove unbound labelled antibodies. On addition of the Substrate (TMB), a colour will develop only in those wells in which enzyme is present, indicating the presence of hGH.

The reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450nm. The concentration of hGH is directly proportional to the colour intensity of the test sample.

This test has been calibrated against hGH WHO 1st International Reference Preparation 65/217

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Microtitre Plate	12 x 8 wells x 1
Breakable wells coated with specific antibodies contained in a resealable foil bag with a desiccant.	
Cal A 0ng/ml	1 ml
Reference Standard: Human serum free of hGH. Ready to use.	
Cal B 2.5ng/ml	1 ml
Reference Standard: hGH diluted in human serum. Ready to use.	
Cal C 5ng/ml	1 ml
Reference Standard: hGH diluted in human serum. Ready to use.	
Cal D 10ng/ml	1 ml
Reference Standard: hGH diluted in human serum. Ready to use.	
Cal E 25ng/ml	1 ml
Reference Standard: hGH diluted in human serum. Ready to use.	
Cal F 50ng/ml	1 ml
Reference Standard: hGH diluted in human serum. Ready to use.	
Conj	11 ml
Anti-hGH HRP Conjugate: Anti-hGH conjugated to Horseradish Peroxidase. Ready to use (Pink)	
Subs TMB	11ml
Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)	
Soln Stop HCl 1M	11ml
Stop Solution: Hydrochloric Acid diluted in purified water. Ready to use. (Colourless)	

Instruction leaflet and EIA Data Recording Sheet 1 + 1

MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes: 100µl, 200µl, and 1000µl
Disposable pipette tips
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME hGH contains materials of human origin which have been tested and confirmed negative for HCV, HIV I and II antibodies and HBsAg by FDA approved methods at single donor level. Because no test can offer complete assurance that products derived from human source will not transmit infectious agents it is recommended that the reagents within this kit be handled with due care and attention during use and disposal. All reagents should, however, be treated as potential Biohazards in use and for disposal. Do not ingest.

PATHOZYME hGH Reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

PATHOZYME hGH Stop Solution is dilute Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

PATHOZYME hGH contain 1% Proclin™ 300* as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with running water and seek medical advice.

*Proclin™ 300 is a trade mark of ROHM & HAAS Limited.

STORAGE

Reagents must be stored at temperatures between 2°C to 8°C.

Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS (except Standards for storage) as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversely affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at -20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

LIMITATIONS OF USE

The use of samples other than serum has not been validated in this test. There is no reuse protocol for this product. When making an interpretation of the test it is strongly advised to take all clinical data into consideration. Diagnosis should not be made solely on the findings of one clinical assay.

ASSAY PROCEDURE

1. Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assay.
2. One set of Standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.
4. Dispense 50µl of Standards and test serum into the assigned wells.
5. Dispense 100µl of Anti-hGH HRP Conjugate into each well. Thoroughly mix for 30 seconds.
6. Incubate the plate for 45 minutes at room temperature (20°C to 25°C).
7. At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the Biohazard container.
8. Hand Washing: Fill the wells with a minimum of 300µl of distilled water per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper.
9. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
10. Machine Washing: Ensure that 300µl of distilled water is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 5 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
11. Dispense 100µl of Substrate Solution into each well and gently mix for 5 seconds.
12. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
13. Stop the reaction by adding 100µl Stop Solution to each well.
14. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
15. Read the optical density immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

TROUBLESHOOTING

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

Use a separate disposable tip for each sample to prevent cross contamination.

Duplication of all standards and specimens, although not required, is recommended.

Specimens and standards should be run at the same time to keep testing conditions the same.

It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used, since pipetting of all Standards and specimens should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available.

Replace caps on all reagents immediately after use.

Avoid repeated pipetting from stock reagents as this is likely to cause contamination. Do not mix reagents or antibody coated strips from different kits. When dispensing, care should be taken not to touch the surface of the well.

Do not allow reagent to run down the sides of the well. Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.

Once an assay has been initiated, the wells should not be allowed to become dry during the assay.

Do not contaminate the Substrate Solution as this will render the whole kit inoperative.

Check the precision and accuracy of the laboratory equipment used during the procedure to ensure reproducible results.

The unused strips should be resealed in the foil bag, containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

CALCULATION OF RESULTS

Calculate the mean absorbance value (A_{550}) for each set of Standards and specimens. Construct a standard curve by plotting the mean absorbance from each Standard against its concentration in ng/ml on graph paper, with absorbance values on the Y-axis and concentrations on the X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of hGH in ng/ml from the Standard Curve.

If levels of controls or users known samples do not give expected results, test results must be considered invalid.

If using a software package choose a quadratic regression curve fit.

EXPECTED VALUES AND SENSITIVITY

The graph produced by the Calibrators should be Hyperbolic in shape with the OD450 of the Calibrators proportional to their concentration. The OD of Calibrator A should be less than 0.75 and the OD of Calibrator F should be greater than 1.5 for the assay results to be valid.

Each laboratory must establish its own normal ranges based on patient populations. A normal range for human growth hormone levels is difficult to define because of the normal physiological fluctuations in hGH concentration. In most adult subjects at rest, after an overnight fast, the hGH level in serum is 7ng/ml or less. Changes in hGH levels in response to various stimuli gives a more accurate assessment of pituitary dysfunction but requires provocative tests, either stimulation or suppression. The minimum detectable concentration of hGH by PATHOZYME hGH test is 0.5ng/ml.

EVALUATION DATA

Calibrated to major competitors and in house standards.
The co-efficient of variation of PATHOZYME hGH is less than or equal to 10%.

In an evaluation of Positive samples between the Omega Pathozyme HGH kit and the Nichols Allegro HGH assay for samples with levels between 0.5 ng/ml and 26.7 ng/ml the following data was generated.

Number of Samples	134
Correlation Co-efficient	0.985
Slope	0.860
Intercept	0.119
Omega Mean	1.78 ng/ml
Nichols Mean	1.65 ng/ml

These kits were shown to give good correlation.

REFERENCES

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4. Reichlin, S. et al. Hypothalamic Hormones. *Annual Review of Medicine* 29:359:1976.
5. Rimoin, D.L. and Horton, W. A. Short Stature. *J. Paediatrics* 92:523:1978.

QUICK REFERENCE TEST PROCEDURE

1. Dispense 50µl of test serum or Standards and 100µl of Anti-hGH HRP Conjugate into each well and mix thoroughly for 30 seconds.
2. Incubate for 45 minutes at room temperature (20°C to 25°C).
3. Discard the well contents and wash 5 times with distilled water.
4. Add 100µl of Substrate Solution to each well. Gently shake for 5 seconds.
5. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
6. Add 100µl Stop Solution to each well and gently shake for 30 seconds.
7. Read Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

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