PATHOZYME® PROLACTIN Ref OD427

Enzyme-Immunoassay (EIA) for the quantitative determination of Prolactin in human serum
Store at 2ºC to 8ºC. DO NOT FREEZE
For in-vitro use only

INTRODUCTION

Human Prolactin (lactogenic hormone) is secreted in both males and females from the anterior pituitary gland. It is a single chain polypeptide hormone with a molecular weight of approximately 23,000 daltons. The release and synthesis of Prolactin is via neuroendocrine control, primarily through Prolactin Releasing Factor and Prolactin Inhibiting Factor.
Women normally have slightly higher basal levels of Prolactin, due to an oestrogen-related rise at puberty and corresponding fall at menopause. Primarily Prolactin functions to initiate breast development and to maintain lactation, although it is also involved in the suppression of gonadal function. During pregnancy, Prolactin levels rise to 10-20 times normal values and decline to non-pregnant levels by 3-4 weeks post-partum. Mothers that breast feed maintain high levels of Prolactin which may take several months to return to non-pregnant levels.
Prolactin concentration determination is useful in the diagnosis of Hypothalamic-Pituitary disorders.
Microadenomas (small pituitary tumours) may cause hyper-prolactinaemia, which is often associated with male impotence. High levels of Prolactin are often associated with galactorrhea and amenorrhea. Prolactin concentrations have been shown to be increased by oestrogens, thyrotropin-releasing hormone (TRH), and several drugs affecting dopaminergic mechanisms. Levels of Prolactin are elevated in renal disease and Hypothyroidism and in some instances associated with stress, exercise and Hypoglycaemia. Additionally, the release of Prolactin is episodic and demonstrates diurnal variation. Slightly elevated levels of Prolactin should be evaluated taking these conditions into account. Prolactin increase may be affected by drugs such as Chloropromazine and Reserpine, and may be decreased by Bromocyptine and L-Dopa.
The following preparations were tested as negative: HCG (WHO 1st International Reference Preparation 75/537) at 500,000 mIU/ml, FSH (WHO 2nd International Reference Preparation HMG) at 500 mIU/ml, LH (WHO 1st International Reference Preparation 68/40) at 1000mIU/ml, TSH (WHO 2nd International Reference Preparation 80/558) 500 mIU/ml and HGH (WHO 1st International Reference Preparation 66/217) at 1000ng/ml.

INTENDED USE

PATHOZYME PROLACTIN is an Enzyme Immunoassay (EIA) for the quantitative determination of Prolactin in human serum.
For professional use only.

PRINCIPLE OF THE TEST

Specific monoclonal anti-Prolactin antibodies are prepared, purified and coated onto microtitration wells. Test sera are applied. Then monoclonal anti-Prolactin labelled with Horseradish Peroxidase enzyme (Conjugate) is added. If the human Prolactin is present in the sample, it will combine with the antibody on the well and the Anti-Prolactin Conjugate, resulting in the Prolactin molecules being %sandwiched+ between the solid phase and the enzyme linked antibodies. After incubation, unbound material is washed away. On addition of the Substrate (TMB), a colour will develop only in those wells in which enzyme is present, indicating the presence of Prolactin.
The reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450nm. The concentration of Prolactin is directly proportional to the colour intensity of the test sample.
This test has been calibrated against in house standards and against the World Health Organisation 1st International Reference Preparation (WHO 1st IRP 75/504).

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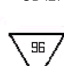

Item	Description	Ref OD427
Microtitre Plate	Breakable wells coated with specific antibodies contained in a resealable foil bag with a desiccant.	12 x 8 wells x 1
Cal A 0 ng/ml	Reference Standard: BSA buffer free of Prolactin. Lyophilised	1 ml
Cal B 5 ng/ml	Reference Standard: Prolactin diluted in BSA buffer. Lyophilised	1ml
Cal C 15 ng/ml	Reference Standard: Prolactin diluted in BSA buffer. Lyophilised	1ml
Cal D 50 ng/ml	Reference Standard: Prolactin diluted in BSA buffer.Lyophilised	1ml
Cal E 100ng/ml	Reference Standard: Prolactin diluted in BSA buffer. Lyophilised	1ml
Cal F 200 ng/ml	Reference Standard: Prolactin diluted in BSA buffer. Lyophilised	1ml
Conj	Anti - Prolactin HRP Conjugate: Anti - Prolactin conjugated to HRP.Ready to use. (Pink)	11ml
Subs TMB	Substrate Solution: 3,3q 5,5qTetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)	11 ml
Soln Stop HCl 1M	Stop Solution: Hydrochloric Acid diluted in purified water. Ready to use. (Colourless)	11ml
	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 PROLACTIN 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 PROLACTIN 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 PROLACTIN Stop Solution is dilute Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

PATHOZYME PROLACTIN reagents contain 1.0% 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 and HAAS Limited.

STORAGE

Reagents must be stored at temperatures between 2ºC to 8ºC.

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 adversly affect the results.

Serum may be stored at 2ºC to 8ºC for up to 48 hours prior to testing. If longer storage is required, store at . 20ºC 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 (20ºC to 25ºC) and mixed gently prior to use. Do not induce foaming.

Add 1ml of distilled water to each Standard vial in order to reconstitute the lyophilised standards. Allow to stand for at least 20 minutes and mix gently. Store at -20°C when not in use. Rehydrated standards can be stored for 30 days at 2°C to 8°C. For long term storage aliquot and freeze at -20°C. Freeze thaw only once. Thawed standards must be mixed prior to testing.

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 assy.
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-Prolactin Conjugate into each well.
6. Mix for 10 seconds. It is very important to mix completely.
7. Incubate the plate for 45 minutes at room temperature (20°C to 25°C).
8. 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.
9. 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. Wash the empty wells 5 times.
10. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.

10. Machine Washing: Ensure that 300μ 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 Substrate Solution into each well and mix gently 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 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 (20ºC to 25ºC). 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 2ºC to 8ºC.

CALCULATION OF RESULTS

Calculate the mean absorbance value (A450) for each set of Standards and test samples. 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 Prolactin 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 greater than 1.5 for the assay results to be valid.
Each laboratory must establish its own normal ranges based on patient population. Based on a limited number of healthy adult blood specimens, the mean Prolactin concentrations for males (N=90) and females (N=120) are estimated to be 6 and 15 ng/ml respectively. The minimum detectable concentration of human Prolactin by **PATHOZYME PROLACTIN** is estimated to be 2 ng/ml.
Concentrations of 4,000 ng/ml have been observed using **PATHOZYME PROLACTIN** with no prozone (Hook) effect.

EVALUATION DATA

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

In an evaluation between the Omega Pathozyme Prolactin kit and the Serono MAIAclone Prolactin Kit for samples with levels between 1.2 and 265.9 ng/ml the following data was generated.

Number of Samples	123
Correlation Co-efficient	0.9802
Slope	1.0493
Intercept	-1.853
Omega Mean	23.27ng/ml
Serono MAIAclone Mean	22.56 ng/ml

These kits were shown to give good correlation.

REFERENCES

1. **Cowden, E. A., Ratcliffe, W. A., Beastall, G. H. and Ratcliffe, J. G.** *Annals Clin. Biochem.* 1979;16:113-121
2. **Frantz, A. G.** *N. Engl. J. Med.* 1978;298:201-207.
3. **Jacobs, L., Snyder, P., Wilber, J., Utiger, R. and Daughaday, W.** *J. Clin. Endocrin.* 1978;33:996.

QUICK REFERENCE TEST PROCEDURE

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

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