

PATHOZYME[®] PROGESTERONE Ref OD487

Enzyme Immunoassay for the quantitative determination of Progesterone in human serum or plasma.

Store at 2°C to 8°C. DO NOT FREEZE.
For in-vitro use only.

INTRODUCTION

Progesterone is a C21 steroid which is synthesised from both tissue and circulating cholesterol. Cholesterol is transformed to pregnenolone which is then converted via a combined dehydrogenase and isomerase to progesterone. The principle production sites are the adrenals and ovaries and the placenta during pregnancy. The majority of this steroid is metabolised in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by the kidneys.

Progesterone exhibits a wide variety of end organ effects. The primary role of progesterone is exhibited by the reproductive organs. In males, progesterone is a necessary intermediate for the production of corticosteroids and androgens. In females, progesterone remains relatively constant throughout the follicular phase of the menstrual cycle. The concentration then increases rapidly following ovulation and remains elevated for 4-6 days and decreases to the initial level 24 hours before the onset of menstruation. In pregnancy, placental progesterone production rises steadily to levels of 10 to 20 times those of the luteal phase peak.

Progesterone measurements are thus performed to determine ovulation as well as to characterise luteal phase defects. Monitoring of progesterone therapy and early stage pregnancy evaluations comprise the remaining uses of progesterone assays.

INTENDED USE

PATHOZYME PROGESTERONE is an Enzyme Immunoassay (EIA) for the quantitative determination of total Progesterone in human serum or plasma.
 For professional use only.

PRINCIPLE OF THE TEST

The **PATHOZYME PROGESTERONE** is based on the principle of competitive binding between Progesterone in the test specimen and Progesterone-HRP Conjugate for a constant amount of rabbit anti-Progesterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with Progesterone standards, controls, patient samples, Progesterone-HRP Conjugate Reagent and rabbit anti-Progesterone Reagent. During the incubation, a fixed amount of HRP-labelled Progesterone competes with the endogenous Progesterone in the standard and sample or quality control serum for a fixed number of binding sites of the specific Progesterone antibody. Thus, the amount of Progesterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of Progesterone in the specimen increases. Unbound Progesterone peroxidase conjugate is then removed and the wells washed. The Substrate (TMB) is then added, resulting in the development of blue colour. The colour development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450nm. The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabelled Progesterone in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The Progesterone concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve. This test has been calibrated against in house standards. There is no International standard for this test.

CONTENTS

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Microtitre Plate	12 x 8 wells x 1
Breakable wells coated with Goat anti Rabbit IgG contained in a resealable foil bag with a desiccant.	
Cal A	0 ng/ml 0.5 ml
Reference Standard: Human serum free of Progesterone. Ready to use. (Colourless)	
Cal B	0.5 ng/ml 0.5 ml
Reference Standard: Progesterone diluted in human serum. Ready to use. (Colourless)	
Cal C	3.0 ng/ml 0.5 ml
Reference Standard: Progesterone diluted in human serum. Ready to use (Colourless)	
Cal D	10 ng/ml 0.5 ml
Reference Standard: Progesterone diluted in human serum. Ready to use (Colourless)	
Cal E	25 ng/ml 0.5 ml
Reference Standard: Progesterone diluted in human serum. Ready to use (Colourless)	
Cal F	50 ng/ml 0.5 ml
Reference Standard: Progesterone diluted in human serum. Ready to use (Colourless)	
Control 1	Level as stated on vial 0.5 ml
Known level of Progesterone diluted in human serum. Ready to use (Colourless)	
Control 2	Level as stated on vial 0.5 ml
Known level of Progesterone diluted in human serum. Ready to use (Colourless)	
Ab REAG Progesterone	7 ml
Rabbit anti Progesterone reagent. Ready to use. (Pink)	
Conj	11X 1.3 ml
Progesterone conjugated to horseradish Peroxidase Ready to use. (Blue)	
DIL CONJ	13 ml
Phosphate based buffer containing stabilising proteins. Ready to use. (Blue)	
Subs TMB	11 ml
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, 1000µl and 5000µl
 Disposable pipette tips
 Absorbent paper
 Microplate reader fitted with a 450nm filter
 Graph paper
 Thoroughly clean laboratory glassware.

PRECAUTIONS

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

PATHOZYME PROGESTERONE reagents contain 1.0% Proclin™ 300* as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with water and seek medical advice.

* Proclin™ 300 is a Trade Mark of ROHM and HAAS Ltd.

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 as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION

Serum:

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.

Plasma:

Obtain a sample of venous blood from the patient and add to EDTA blood collection vial. Centrifuge sample and collect clear plasma. Fresh plasma samples are required.

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

Serum or plasma 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.

Working Solution: Dilute the concentrated conjugate using 1 part conjugate to 10 parts conjugate diluent (1/11 dilution) 100µl is required per well. Diluted reagent is stable at 2°C to 8°C for one month.

LIMITATIONS OF USE

The use of samples other than serum and EDTA plasma have 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

- Bring all the kit components and the test sample to room temperature (20°C to 25°C) prior to the start of the assay.
- One set of Standards should be run with each batch of test sample. Secure the desired number of coated wells in the holder. Record the position of the standards and the test samples on the EIA Data Recording Sheet provided.
- 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.
- Dispense 25µl of standards, test samples and controls into the appropriate wells.
- Dispense 100µl working solution of Progesterone-HRP Conjugate Reagent to each well.
- Dispense 50µl of rabbit anti-Progesterone Reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
- Incubate at room temperature (20°C to 25°C) for 90 minutes.
- 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.
- 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.
- Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
- 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.
- Dispense 100µl of Substrate solution into each well. Gently mix for 5 seconds.
- Incubate in the dark at room temperature (20°C to 25°C) for 20 minutes.
- Stop the reaction by adding 100µl of Stop Solution to each well.
- Gently mix for 30 seconds. It is important to make sure that all the blue colour changes to yellow colour immediately.
- Read the absorbance at 450nm with a microtitre well reader within 10 minutes.

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_{450}) for each set of Standards, Controls and samples. Construct a point to point standard curve by plotting the mean absorbance obtained for each Standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y-axis and concentrations horizontal or the X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of Progesterone 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 polygon with data extrapolation curve fit.

EXPECTED VALUES AND SENSITIVITY

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

Each laboratory should establish its own normal range based on the patient population.

PATHOZYME PROGESTERONE was performed on randomly selected outpatient clinical laboratory samples.

The results of these determinations are as follows:

Males	Adult	0.13-0.97ng/ml
	Prepubertal (children)	0.07-0.52g/ml
Females	Follicular phase	0.15- 0.70ng/ml
	Luteal phase	2.00-25.0ng/ml
	Post menopausal	0.06-1.60ng/ml
	Pregnancy	
	1 st Trimester	10.3 - 44.0 ng/ml
2 nd Trimester	19.5 - 82.5 ng/ml	
3 rd Trimester	65.0 - 229.0 ng/ml	

SENSITIVITY

The lowest detectable level of Progesterone in this test is approximately 0.0625ng/ml

SPECIFICITY

The following materials have been checked for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Progesterone.

Data on the cross-reactivity for several endogenous and pharmaceutical steroids are summarised in the following table:

Cross-reactivity(%) = $\frac{\text{Observed Progesterone Concentration} \times 100}{\text{Steroid Concentration}}$

Steroid	Cross-reactivity
Progesterone	100%
Androsterone	0.086%
Corticosterone	0.74%
Cortisone	0.11%
Cholesterol	<0.08%
Oestradiol	<0.01%
Estrone	0.08%
Estrilol	<0.024%
Prednisolone	0.075%
Testosterone	0.1%

EVALUATION DATA

Calibrated to major competitors and in house standards.

The co-efficient of variation of **PATHOZYME PROGESTERONE** is less than or equal to 10%

In an evaluation between the Omega Pathozyme Progesterone kit and the DRG BIOC Kit for samples with levels between 0.35 ng/ml and 73.83 ng/ml the following data was generated.

Number of Samples	106
Correlation Co-efficient	0.96
Slope	1.16
Intercept	1.53
Omega Mean	18.0 ng/ml
DRG Mean	14.17 ng/ml

These kits were shown to give good correlation.

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QUICK REFERENCE TEST PROCEDURE

- Dispense 25µl of standards, test samples, controls.
- Add 100µl working solution Progesterone HRP conjugate to each well.
- Add 50µl of Rabbit anti-Progesterone into each well. Gently mix for 30 seconds.
- Incubate for 90 minutes at room temperature (20°C to 25°C)
- Discard the well contents and wash 5 times with distilled water.
- Add 100µl of substrate solution into each well and gently mix for 5 seconds.
- Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
- Add 100µl Stop Solution to each well and gently mix for 30 seconds.
- Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

8098 ISSUE 5 Revised March 2010

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