PATHOZYME® OESTRADIOL Ref OD477

Enzyme-Immunoassay (EIA) for the quantitative determination of Oestradiol in human serum or plasma.

Store at 2°C to 8°C. DO NOT FREEZE

For in-vitro use only.

INTRODUCTION

Oestradiol (E2) is a C18 steroid human hormone with a phenolic A ring . This steroid hormone has a molecular weight of 272.4 daltons. It is the most potent natural Oestrogen, produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex, and the male testes[1, 2, 3].
Oestradiol (E2) is secreted into the blood stream where 98% of it circulates bound to sex hormone binding globulin (SHBG). To a lesser extent it is bound to other serum proteins such as albumin. Only a tiny fraction circulates as free hormone or in the conjugated form[4, 5]. Oestrogenic activity is affected via Oestradiol-receptor complexes which trigger the appropriate response at the nuclear level in the target sites. These sites include the follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and to a lesser extent, the liver and skin.
In non-pregnant women with normal menstrual cycles, Oestradiol secretion follows a cyclic, biphasic pattern with the highest concentration found immediately prior to ovulation[6, 7]. The rising Oestradiol concentration is understood to exert a positive feedback influence at the level of the pituitary where it influences the secretion of the gonadotropins, follicle stimulating hormone (FSH) and luteinising hormone (LH), which are essential for follicle maturation and ovulation, respectively[8, 9]. Following ovulation, Oestradiol levels fall rapidly until the luteal cells become active resulting in a secondary gentle rise and plateau of Oestradiol in the luteal phase. During pregnancy, maternal serum Oestradiol levels increase considerably to well above the pre-ovulatory peak levels and high levels are sustained throughout pregnancy[10].
Serum Oestradiol measurements are a valuable index in evaluating a variety of menstrual dysfunctions such as precocious or delayed puberty in girls[11] and primary and secondary amenorrhea and menopause[12]. Oestradiol levels have been reported to be increased in patients with feminising syndromes[14], gynaecomastia[15] and testicular tumours[16].
In cases of infertility, serum Oestradiol measurements are useful for monitoring induction of ovulation following treatment with, for example, clomiphene citrate, LH-releasing hormone (LH-RH), or exogenous gonadotropins[17, 18]. During ovarian hyperstimulation for in vitro fertilisation (IVF), serum Oestradiol concentrations are usually monitored daily for optimal timing of human chorionic gonadotropin (hCG) administration and oocyte collection[19].

INTENDED USE

PATHOZYME OESTRADIOL (E2) is an Enzyme Immunoassay (EIA) for the quantitative determination of total Oestradiol (E2) in human serum or plasma.
For professional use only.

PRINCIPLE OF THE TEST

PATHOZYME OESTRADIOL (E2) is based on the principle of competitive binding between (E2) in the test specimen and (E2)-HRP Conjugate for a constant amount of rabbit anti-Oestradiol. In the incubation, goat anti-rabbit IgG-coated wells are incubated with (E2) standards, controls, patient samples, Oestradiol-HRP Conjugate Reagent and rabbit anti-Oestradiol Reagent. During the incubation, a fixed amount of HRP-labelled (E2) competes with the endogenous (E2) in the standard, sample, or quality control serum for a fixed number of binding sites of the specific (E2) antibody. Thus, the amount of (E2) peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of (E2) in the specimen increases.
Unbound (E2) peroxidase conjugate is then removed and the wells washed. A solution of 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 (E2) in the sample.
A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The (E2) 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

Ref
OD477

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 pg/ml — **0.5 ml**
Reference Standard: Human serum free of Oestradiol.
Ready to use. (Colourless)

Cal | B | 10 pg/ml — **0.5 ml**
Reference Standard: Oestradiol diluted in human serum.
Ready to use. (Colourless)

Cal | C | 30 pg/ml — **0.5 ml**
Reference Standard: Oestradiol diluted in human serum.
Ready to use (Colourless)

Cal | D | 100 pg/ml — **0.5 ml**
Reference Standard: Oestradiol diluted in human serum.
Ready to use (Colourless)

Cal | E | 300 pg/ml — **0.5 ml**
Reference Standard: Oestradiol diluted in human serum.
Ready to use (Colourless)

Cal | F | 1000 pg/ml — **0.5 ml**
Reference Standard: Oestradiol diluted in human serum.
Ready to use (Colourless)

Control | 1 | Level as stated on vial — **0.5 ml**
Known level of Oestradiol diluted in human serum.
Ready to use (Colourless)

Control | 2 | Level as stated on vial — **0.5 ml**
Known level of Oestradiol diluted in human serum.
Ready to use (Colourless)

Ab | REAG | Oestradiol — **7 ml**
Rabbit anti Oestradiol reagent.
Ready to use. (Pink)

Conj — **11 ml**
Oestradiol conjugated to horseradish Peroxide
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 and 1000µl
Disposable pipette tips
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME OESTRADIOL 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. Do not ingest. All reagents should, however, be treated as potential Biohazards in use and for disposal.

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

PATHOZYME OESTRADIOL 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 adversly 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.

LIMITATIONS OF PROCEDURE

The use of samples other than serum or 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

1. Bring all the kit components and the test samples 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 sample. Secure the desired number of coated wells in the holder. Record the position of the standards and the test sample 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.Secure the desired number of coated wells in the holder.
4. Dispense 25µl of Standards and test sample into the appropriate wells.
5. Dispense 100µl of Oestradiol-HRP Conjugate reagent into each well.
6. Dispense 50µl of rabbit anti-Oestradiol (E2) Reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
7. Incubate at room temperature (20ºC to 25ºC) for 90 minutes.
8. Hand Washing: 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. 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.
11. 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.
12. Dispense 100µl Substrate Solution into each well. Gently mix for 5 seconds.
13. Incubate in the dark at room temperature (20ºC to 25ºC) for 20 minutes.
14. Stop the reaction by adding 100µl Stop Solution to each well.
15. Gently mix for 30 seconds. It is important to make sure that all the blue colour changes to a yellow colour immediately.
16. 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 from each Standard against its concentration in pg/ml as point to point on linear-linear 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 Oestradiol in pg/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 indirectly proportional to their concentration. The OD of Calibrator A should be greater than 1.5 and the OD of Calibrator F should be 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 OESTRADIOL (E2)** was performed on randomly selected outpatient clinical laboratory samples.

The results of these determinations are as follows:

Males:	Adult	<60 pg/ml
Females:	Post menopausal	<18 pg/ml
	Ovulating, early follicular	30-100 pg/ml
	Late follicular	100-400 pg/ml
	Luteal phase	60-150 pg/ml
	Pregnant, normal	up to 35,000 pg/ml
	prepubertal children normal	<10 pg/ml

SENSITIVITY

The lowest detectable level of Oestradiol in this test is 1pg/ml.

SPECIFICITY

The following materials have been checked for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Oestradiol (E2).
Data on the cross-reactivity for several endogenous pharmaceutical steroids are summarised in the following table:

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

Steroid	Cross-reactivity
Oestradiol	100%
Oestrone	2.10%
Oestriol	1.50%
17a Oestriol	0.30%
Cortisol	<0.01%
Cortisone	<0.01%
Progesteron	<0.01%
Testosterone	<0.01%
DHEA-Sulphate	<0.01%
5a Dihydrotestosterone	<0.01%

EVALUATION DATA

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

In an evaluation between the Omega Pathozyme Oestradiol kit and the DRG Oestradiol Kit for samples with levels between 17 and 1351 pg/ml the following data was generated.

Number of Samples	174
Correlation Co-efficient	0.98
Slope	1.05
Intercept	- 3.67
Omega Mean	367 pg/ml
DRG Mean	279 pg/ml

These kits were shown to give good correlation.

REFERENCES

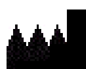
(1) **Tsang, B. K., Armstrong, D. T. and Whitfield, J. F.** Steroid Biosyntheses by isolated human ovarian follicular cells in vitro. *J. Clin. Endocrinol. Metab.* 51:1407-1411;1980.
(2) **Gore-Langton, R.E. and Armstrong, D. T.** Follicular steroidogenesis and its control. In: The Physiology of Reproduction, Ed. Knobil, E. and Neill, J. et al. p331-385. Raven Press, New york. 1988.
(3) **Hall, P. F.** Testicular Steroid Synthesis: Organisation and Regulation. In: The Physiology of Reproduction, Ed. Knobil, E. and Neill, J. et al. p975-998. Raven Press, New York. 1982.
(4) **Siiteri, P.K.., Murai, J. T., Hammon, G.L., Nisker, J. A., Raymoure, E. J. and Kuhn, R. W.** The serum transport of steroid hormones. *Rec. Prog. Horm. Res.* 38:457-510;1988.
(5) **Martin, B., Rotten, D., Jolivet, A. and Gautray, J. P.** Binding of steroids by proteins in follicular fluid of the human ovary. *J. Clin. Endocrinol. Metab.* 35:443-447;1981.
(6) **Bard, D. T.** Ovarian steroid secretion and metabolism in women. In: The Endocrine Function of the Human Ovary. Eds. James, V.H.T., Serio, M. and Giusti, G. p125-133. Academic Press, New York. 1976.
(7) **McNastty, K. P., Baird, D. T., Bolton, A., Chambers, P., Corker, C. S. and McLean, H.** Concentration of oestrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. *J. Endocrinol.* 71:77-85;1976.
(8) **Abraham, G. E., Odell, W. D., Swerdloff, R. S. and Hopper, K.** Simultaneous radioimmunoassay of plasma FSH, LH, progesterone, 17-hydroxyprogesterone and oestradiol-17B during the menstrual cycle. *J. Clin. Endocrinol. Metab.* 34:312-318;1972.
(9) **March, C. M., Goebelsmann, U., Nakumara, R. M. and Mishell, D. R**. Roles of Oestradiol and progesterone in eliciting midcycle luteinising hormone and follicle stimulating hormone surges. *J. Clin. Endocrinol.* 49:507-512;1979.
(10) **Simpson, E. R. and McDonald, P.C.** Endocrinology of Pregnancy. In: Textbook of Endocrinology, Ed. Williams, R. H. p412-422. Saunders Company, Philadelphia. 1981.
(11) **Jenner, M. R., Kelch, R. P.** et al. Hormonal changes in prepubertal children, pubertal females in precocious puberty, premature thelarche, hypergonadoism and in a child with feminising tumour. *J. Clin. Endocrinol.* 34:521;1982.
(12) **Goldstein, D.** et al. Correlation between oestradiol and progesterone in cycles with luteal phase deficincy. *Fertil. Steril.* 37: 348-354;1982.
(13) **Kirschner, M. A.** The role of hormones in the etiology of human breast cancer. *Cancer.* 39:2716-2726;1977.
(14) **Odell, W. D. and Swerdloff, R. D.** Abnormalities of gonadal function in men. *Clin. Endocrinol.* 8:149-180;1978.
(15) **McDonald, P.C., Madden, J.C., Brenner, P.F., Wilson, J. D. and Siiteri, P. K.** Origin of oestrogen in normal men and women with testicular feminisation. *J. Clin. Endocrinol. Metabl.* 49:905;1979.
(16) **Peckham, M. J. and McElwain, T. J.** Testicular tumours. *J. Clin. Endocrinol. Metab.*
(17) **Taubert, H. D. and Dericks-Tan, J. S. E.** Induction of ovulation by clomiphene citrate in combinatiuon with high doses of estrogens or nasal application of LH-RH. In: Ovulation in the Human. Eds. Crosignandi, P. G. and Mishell, D. R. p265-273. Academic Press, New York. 1976.
(18) **Fishel, S. B., Edwards, R. G, Purdy, J. M., Steptoe, P.C., Webster, J., Walters, E., Cohen, J., Fehilly, C., Hewitt, J. and Rowland, G.** Implantation, abortion and birth after in-vitro stimulation with clomiphene citrate and human menopausal gonadotropin. *J. In Vitro Fertil. Embryo Transfer.* 1:24-28;1985.
(19) **Wramsby, H., Sundstrom, P. and Leidholm, P.** Pregnancy rate in relation to number of cleaved eggs replaced after in-vitro fertilisation of stimulating cycles monitored by serum levels of estradiol and progesterone as sole index. *Human Reproduction.* 2:235-328;1987.
(20) **Ratcliffe, W. A., Carter, G. D.** et al. Estradiol assays: applications and guidelines for the provision of clinical biochemistry service. *Ann. Clin. Biochem.* 25:466-483;1988.
(21) **Tietz, N. W.** Textbook of Clinical Chemistry. Saunders. 1986.
(22) USA Centre for Disease Control/National Institute of Health Manual. **Biosafety in Microbiological and Biomedical Laboratories.** 1984.

QUICK REFERENCE TEST PROCEDURE

1. Dispense 25µl of standards, test sample, controls into each well.
2. Add 100µl Oestradiol HRP conjugate to each well.
3. Add 50µl of Rabbit anti-Oestradiol into each well. Gently mix for 30 seconds.
4. Incubate for 90 minutes at room temperature (20ºC to 25ºC).
5. Discard the well contents and wash five times with distilled water.
6. Add 100µl Substrate Solution to each well and shake for 5 seconds.
7. Incubate in the dark for 20 minutes at room temperature (20ºC to 25ºC).
8. Add 100µl of Stop Solution to each well and gently mix for 30 seconds.
9. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

8097 ISSUE 4A Revised October 2006

© Omega Diagnostics Ltd., 2006



OMEGA DIAGNOSTICS LTD.
Omega House, Hillfoots Business Village
Alva FK12 5DQ, Scotland, United Kingdom
odl@omegadiagnostics.co.uk
www.omegadiagnostics.com
AN ISO 9001 AND ISO 13485 CERTIFIED COMPANY