EIASON® Estradiol sensitive

IVD

Enzymeimmunoassay for the quantitative determination of Estradiol in human serum or plasma

Kit instruction

For in-vitro use only

Product of

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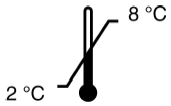
REF E02-006-96

2 °C 8 °C

CE

Used IFU-Symbols

Symbol	English	Symbol	English
IVD	In vitro diagnostic device	SUB	Substrate
REF	Order number	CAL 0-4	Calibrators
	Product of	STOP	Stop solution
	Storage	WASH	Wash Buffer
CE	European Conformity	LOT	Batch code
	Expiry date	DIL	Sample Diluent
CONJ	Conjugate	MPL	Microplate

Intended use

For in-vitro use only.
The EIASON® Estradiol sensitive ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of Estradiol in serum and plasma

Summary

Estradiol (1,3,5(10)-estratriene-3,17β-diol; 17β-estradiol; E2) is a C18 steroid hormone with a phenolic A ring. This steroid hormone has a molecular weight of 272.4. It is the most potent natural Estrogen, produced mainly by the Graffian follicle of the female ovary and the placenta, and in smaller amounts by the adrenals, and the male testes.
Estradiol (E2) is secreted into the blood stream where 98% of it circulates bound to sex hormone binding globulin (SHBG) and to a lesser extent to other serum proteins such as albumin. Only a small fraction circulates as free hormone or in the conjugated form.

Estrogenic activity is affected via estradiolreceptor complexes which trigger the appropriate response at the nuclear level in the target sites. These sites include the follicles, uterus, breast, vagine, urethra, hypothalamus, pituitary and to a lesser extent the liver and skin. In non-pregnant women with normal menstrual cycles, estradiol secretion follows a cyclic, biphasic pattern with the highest concentration found immediately prior to ovulation.
The rising estradiol 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 folicular maturation and ovulation, respectively.
Following ovulation, estradiol levels fall rapidly until the luteal cells become active resulting in a secondary gentle rise and plateau of estradiol in the luteal phase. During pregnancy, maternal serum Estradiol levels increase considerably, to well above the pre-ovulatory peak levels and high levels are sustained throughout pregnancy.
Serum Estradiol measurements are a valuable index in evaluating a variety of menstrual dysfunctions such as precocious or delayed puberty in girls and primary and secondary amenorrhea and menopause.
Estradiol levels have been reported to be increased in patients with feminising syndromes, gynaecomastia and testicular tumors.
In cases of infertility, serum Estradiol measurements are useful for monitoring induction of ovulation following treatment with, for example, clomiphene citrate, LH-releasing hormone (LH-RH), or exogenous gonadotropins. During ovarian hyperstimulation for in vitro fertislisation (IVF), serum estradiol concentrations are usually monitored daily for optimal timing of human chorionic gonadotropin (hCG) administration and oocyte collection.

Assay principle

The EIASON® Estradiol sensitive ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.
The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the Estradiol molecule. Endogenous Estradiol of a patient sample competes with an Estradiol horseradish peroxidise conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is reverse proportional to the concentration of Estradiol in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of Estradiol in the patient sample.

Warnings and precautions

The EIASON® Estradiol sensitive ELISA Kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IASON will not be held responsible for any loss or damage (except as required by statute) caused, arising out of non-compliance with the instructions provided.
CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.
Source material from Human origin which is used in this kit was tested and found negative for HbsAG and HIV as well as for HCV antibodies. However, since there is

no diagnostic procedure that excludes these agents with 100 percent certainty all components should be handled as potentially hazardous material.
Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Shelf life and storage of reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C.
Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above

Storage and preparation of serum samples

Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.
Do not use lipaemic or grossly haemolytic or icteric specimens.

Serum:
Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.
Plasma:
Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.
Storage:
Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. When required, thaw test sera at room temperature and mix gently to ensure homogeneity.
Specimen Dilution:
If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with DIL and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µl Serum + 90 µl DIL (mix thoroughly)
b) Dilution 1:100: 10 µl dilution a) 1:10 + 90 µl DIL (mix thoroughly).

Materials provided

Allow all reagents 1-7 to reach room temperature before use.

1. MPL ELISA strip wells coated with an anti-Estradiol antibody (polyclonal). (96 wells in total, 8 wells per strip). Before opening the packet of strip wells, allow it to stand at room temperature (20-25°C) for at least 30 minutes. After opening, keep any unused wells in the original foil packet (reseal with adhesive tape) and in the self-seal plastic bag with the desiccant provided. Store at 2-8°C and use within 2 months;
2. CAL 0-4 Calibrators(1 mL each); ready to use:
   Concentrations: 0; 3; 10; 50; 200 pg/mL
   Conversion: 1 pg/mL = 3.67 pmol/l;
3. DIL Sample diluent 1 vial, 3 mL, ready to use
4. WASH Wash solution (30 mL, 40 x concentrated);
   dilute to 1.2 L with distilled water before use. The diluted WASH is stable for 2 weeks at room temperature;
5. CONJ Enzyme Conjugate 1 vial, 25 mL, ready to use
   Estradiol conjugated to horseradish peroxidase;
6. SUB Peroxidase substrate (tetramethyl benzidine; TMB; 25 mL ready to use);
7. STOP Stop solution (0.5 M sulphuric acid; 14 mL ready to use);

Materials required but not provided in the kit

- Pipettes capable of dispensing 100 µL and 200 µL
- Distilled water
- ELISA plate reader suitable for 96 well formats and capable of measuring absorbances at 450 and 405 nm

Assay procedure

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming. Once the test has been started, all steps should be completed without interruption.
As a general rule the enzymatic reaction is linearly proportional to time and temperature.
Calculate the number of individual MPL wells needed for the assay. Allow all the reagents supplied including the appropriate number of strips to reach room temperature, fit the number of strip wells required firmly into the frame provided.

Controls and a standard curve should always be included in each assay run.

1. Pipette 100 µL of CAL 0-4, controls and test sera into the wells (in duplicate).
2. Pipette carefully 200 µL of CONJ into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step
3. Incubate for 4 hour at 20-25°C.
4. After the incubation with CONJ, discard the CONJ by briskly inverting the wells over a suitable receptacle, wash 3 times with 400µL diluted WASH per well and each time tap the inverted wells gently on a clean dry absorbent surface to remove any droplets of wash buffer.
5. Pipette carefully 200 µL of SUB into each well.
6. Incubate for 30 minutes at 20-25°C in the dark during which time a blue colour will develop.
7. Stop the substrate reaction by careful addition of 100 µL of STOP to each well (this will cause the blue colour to turn yellow) and shake the plate for about 5 seconds on a plate shaker to ensure uniformity of the solution in each well. It is most important to ensure that the substrate incubation time (i.e. time from addition of SUB to addition of STOP) is the same for each well.
8. Measure the absorbance of each well at 450 nm as reading filter (and 405 nm as overrange filter if possible) (reference 620 – 650 nm) within 10 minutes after adding the stop solution.

or fully automated on

- **IASON® Quardette**
- **IASON® PersonalLab**
- **IASON® Gladiator**

Quality control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance.
Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or IASON directly.

Calculation of results

- Draw a standard curve by plotting the absorbance of each standard against its concentration. Read off the values of the test samples. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.
- Alternative data reduction techniques may be employed but users should confirm that the selected curve fit is appropriate and gives acceptable results. 4PL (4 parameter logistics) or point-to-point fits are recommended.

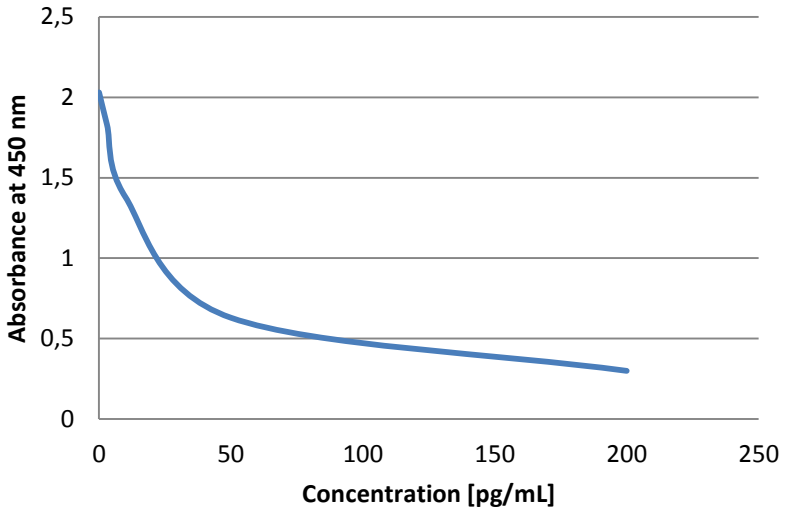
Sample assay data

Example of typical results obtained with EIASON® Estradiol calibrators:

Calibrator [pg/mL]	Absorbance at 450 nm
0	2.03
3	1.83
10	1.38
50	0.63
200	0.30

This data is for illustration only and must not be used for the calculation of any sample result.

Typical calibration curve



This sample calibration curve is for illustration only.

Expected Values

	5-95% percentile
	pg/mL
Males	10 - 36
Females	
Pre-menopausal	13 – 191
Post-menopausal	11 - 65

Each laboratory is recommended to determine ranges for their local population. The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests

ASSAY CHARACTERISTICS

Assay dynamic range

The range of the assay is between 0-200 pg/mL.

Cross-reactivity

Steroid	Cross-reactivity %	Steroid	Cross-reactivity %
Estradiol	100	11-Desoxycorticosterone	0
Estron	0.2	21-Desoxycortisol	0
Estriol	0.05	Dihydrotestosterone	0
Androstenedione	0	Dihydroepiandrosterone	0
Androsterone	0	20-Dihydroprogesterone	0
Corticosterone	0	11-Hydroxyprogesterone	0
Epiandrosterone	0	17a-Hydroxyprogesterone	0
16-Epiestriol	0	17a-Pregnenolone	0

Estradiol-3-sulfate	0	17a Progesterone	0
Estradiol-3-glucuronide	0	Pregnanediol	0
Estradiol-17?	0	Pregnantriol	0
Estriol-16-glucuronide	0	Pregnenolone	0
Estrone-3-sulfate	0	Progesterone	0
Dehydroepiandrosterone	0	Testosterone	0
11-Desoxycortisol	0		

Analytical sensitivity: < 1.4 pg/mL

The analytical sensitivity was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of CAL 0.

Precision

Intra-assay-precision (n=20)			Inter-assay-precision (n=12)		
sample	**Mean [pg/mL]**	**CV [%]**	**sample**	**Mean [pg/mL]**	**CV [%]**
A	8.21	7.87	A	7.87	8.78
B	18.50	5.68	B	17.87	7.25
C	27.62	5.52	C	26.71	6.78

Recovery

Samples have been spiked by adding Estradiol solutions with known concentrations in a 1:1 ratio. The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Added conc. 1:1 (v/v) [pg/mL]	Measured conc. [pg/mL]	Expected conc. [pg/mL]	Recovery [%]
A	0	58.30		
	100.0	116.58	129.15	90.3
	25.0	55.19	54.15	101.9
	5.0	32.70	34.15	95.7
	1.5	26.65	30.65	87.0
B	0	19.07		
	100.0	95.71	109.54	87.4
	25.0	35.02	34.54	101.4
	5.0	16.52	14.54	113.7
	1.5	12.52	11.04	113.4
C	0	78.22		
	100.0	158.73	139.11	114.1
	25.0	69.43	64.11	108.3
	5.0	37.93	44.11	86.0
	1.5	34.72	40.61	85.5

Linearity

Sample	Dilution	Measured conc. [pg/mL]	Expected conc. [pg/mL]	Recovery [%]
A	None	50.64	50.64	
	1:2	23.01	25.32	90.9
	1:4	12.83	12.66	101.3
	1:8	7.23	6.33	114.3
B	None	20.68	20.68	
	1:2	11.47	10.34	110.9
	1:4	5.82	5.17	112.6
	1:8	2.95	2.59	114.3
C	None	65.74	20.68	
	1:2	29.46	32.87	89.6
	1:4	15.10	16.44	91.8
	1:8	8.16	8.22	99.2

Interferences

Any improper handling of samples or modification of this test might influence the results.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Estradiol in a sample. No high-dose-hook effect was observed in this test.

LEGAL ASPECTS

Reliability of results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IASON.

Therapeutic consequences

Therapeutic consequences should never be based on laboratory results only even if all test results are in agreement with the items as stated under Reliability of Results. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point Therapeutic Consequences are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

Useful publications

1. Tsang, B.K., Armstrong, D.T. and Whitfield, J.F., Steroid biosyntheses by isolated human ovarianfollicular cells in vitro, J. Clin. Endocrinol. Metab. 51:1407 - 11 (1980).
2. Gore-Langton, R.E. and Armstrong, D.T., Follicular stoidogenesis and its control. In: The physiology of Reproduction, Ed.: Knobil, E., and Neill, J. et al., pp. 331-85. Raven Press, New York (1988).
3. Hall, P.F., Testicular Steroid Synthesis: Organization and Regulation. In: The Physiology of Reproduction, Ed.: Knobil, E., and Neill, J. et al., pp 975-98. Raven Press, New York (1988).
4. Siiteri, P.K. Murai, J.T., Hammond, G.L., Nisker, J.A., Raymoure, W.J. and Kuhn, R.W., The serum transport of steroid hormones, Rec. Prog. Horm. Res. 38:457 - 510 (1982).
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. Endicrinol. Metab. 35: 443-47 (1981).
6. Baird, 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. pp. 125-33, Academic Press, New York (1976).
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8. Abraham, G.E., Odell, W.D., Swerdloff, R.S., and Hopper, K., Simultaneous radioimmunoassay of plasma FSH, LH, progesterone, 17-hydroxyprogesterone and estradiol-17ß during the menstrual cycle, J.Clin. Endocrinol. Metab. 34:312-18 (1972).
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10. Simpson, E.R., and McDonald, P.C., Endocrinology of Pregnancy. In: Textbook of Endocrinology, Ed.: Williams, R.H. pp412-22, Saunders Company, Philadelphia (1981).
11. Jenner, M.R., Kelch, R.P., et al., Hormonal Changes in prepubertal children, pubertal females and in precocious puberty, premature thelarche, hypogonadism 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 deficiency, Fertil. Steril. 37: 348-54 (1982).
13. Kirschner, M.A., therole of hormones in the etiology of human breast cancer, Cancer 39:2716 26 (1977).
14. Odell, W.D. and Swerdloff, R.D., Abnormalities of gonadal function in men, clin. Endocr. 8:149-80 (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. Endcrinol. Metabol. 49:905 (1979).
16. Peckham, M.J: and McElwain, T.J:, Testicular tumours, J.Clin. Endocrinol. Metab. 4:665-692 (1975).
17. Taubert, H.d. and Dericks-Tan, J.s.E., Induction ofr ovulation by clomiphene citrate in combination with high doses of oestrogens or nasal application of LH-RH. In: Ovulation in the Human. Eds.: Crosignandi, P.G. and Mishell, D.R., pp.265-73, Academic Press, New York (1976).
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19. Wramsby, H., Sundstorm, 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 oestradiol and progesterone as sole index. Human Reproduction 2: 325-28 (1987).
20. Ratcliff, 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.

Pipetting scheme

Allow all reagents to reach room temperature before use.

1. Pipetting	CAL 0-4 **100 µL**	Controls **100 µL**	Samples **100 µL**
2. Pipetting	CONJ **200µL** Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step		
3. Incubation	4 hours at room temperature (20-25°C)		
4. Washing	wash 3 x : aspirate or decant add 400 µL WASH aspirate or decant and dry on an absorbent material		
5. Pipetting	SUB **200µL**		
6. Incubation	30 min at room temperature (20-25°C)		
7. Pipetting	STOP **100µL**		
8. Reading	450nm (RF 620 – 650nm) Optional Overrange Filter: 405nm, Factor: 3 (dependent on photometer), reading within 10 min. Calculation: 4-parameter or point to point		

Expected Values

	5-95% percentile
	pg/mL
Males	10 - 36
Females	
Pre-menopausal	13 – 191
Post-menopausal	11 - 65