Diagnostics Biochem Canada Inc.
Manufacturing Innovative IVD for the World Since 1973

TOTAL ESTROGENS ELISA

EU: CE IVD CAN: IVD USA: IVD

REF: CAN-E-440

Version: 5.0
Effective: September 14, 2018

INTENDED USE

For the direct quantitative determination of Total Estrogens in human serum by an enzyme immunoassay. For *in vitro* use only.

PRINCIPLE OF THE TEST

The total estrogens ELISA is a competitive immunoassay. Competition occurs between total estrogens (estrone, estradiol, and estriol) present in standards, controls and patient samples and an enzyme-labelled antigen (conjugate) for a limiting number of anti-estrogen antibody binding sites on the microplate wells. After a washing step that removes unbound materials the enzyme substrate is added and approximately 15–20 minutes later the enzymatic reaction is terminated by addition of stopping solution. The resulting optical density (OD), measured with a microplate reader, is inversely proportional to the concentration of total estrogens in the sample. A standard curve is plotted with a provided set of standards to calculate directly the concentration of total estrogens in patient samples and controls.

CLINICAL APPLICATIONS

Total estrogens comprise the total quantity of estrone, estradiol, and estriol. The estrogens are involved in the development of female sex organs and secondary sex characteristics. Before the ovum is fertilized the main action of the estrogens is on the growth and function of the reproductive tract to prepare it for the fertilized ovum.

During the follicular phase of the menstrual cycle the total estrogens level shows a slight increase. The production of total estrogens then increases markedly to peak at around day 13. The peak is of short duration and by day 16 of the cycle levels will be low. A second peak occurs at around day 21 of the cycle; if fertilization does not occur, the production of total estrogens decreases.

In post-menopausal women the concentration of all estrogens decreases substantially and estrone becomes the predominant estrogen. In pregnant women the concentration of all estrogens escalates and estriol becomes the predominant estrogen.

A total estrogens test is commonly indicated to:

- Aid in diagnosis of sex steroid metabolism related conditions, for example, premature or delayed puberty, and aromatase and 17 alpha-hydroxylase deficiencies.
- Assess fracture risk in postmenopausal women and, to a lesser degree, older men.
- Follow-up female hormone replacement therapy in postmenopausal women.
- Prognose antiestrogen therapy, for example, aromatase inhibitor therapy.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is intended for in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of the kit. Reliable performance will only be attained by strict and careful adherence to the instructions.
4. Include control materials or serum pools in every run at a high and low level to assess the reliability of results.
5. Use deionized or distilled water to dilute wash buffer concentrate.
6. Wear gloves to handle kit reagents and human specimens and reduce exposure to potentially harmful substances.
7. Bring the microplate, kit reagents, and specimens to room temperature and mix them gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
8. Establish a calibrator curve for every run.
9. Include provided controls in every run and corroborate that they fall within established quality control certificate limits.
10. Follow good laboratory practices. Improper procedural techniques, imprecise pipetting, incomplete washing, as well as improper reagent storage may be the cause of kit controls not falling within established limits.
11. Carefully remove bubbles before reading. The presence of bubbles in the microplate wells can affect the OD.
12. Do not use the substrate (TMB) if it is blue before the test. The TMB solution shall remain colourless if stored under recommended conditions (see label). Exposure to light or contamination might turn it blue.
13. Do not use pipettes in which liquids contact metal parts.
14. Use a new disposable pipette tip for dispensing each reagent, sample, standard and control to prevent contamination of reagents.
15. Do not mix components from various kit lot numbers within a test and do not use any component beyond the expiration date printed on the label.
16. Dispose leftover kit reagents according to national regulations as they may be considered hazardous waste.

LIMITATIONS

1. This kit is calibrated for the direct determination of total estrogens in human serum; not for the determination of total estrogens in other species or in specimens other than serum.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Samples or control sera containing azide or thimerosal are not compatible with this kit, they may lead to false results.
4. Only calibrator A may be used to dilute high serum samples. The use of any other reagent (including water) will lead to false results.
5. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis. For example, some drugs and heterophilic antibodies in patients regularly exposed to animals or animal products have the potential to interfere with immunological tests. Consequently, the clinical diagnosis should comprise all aspects of a patient's background including the frequency of exposure to animals/products.

SAFETY CAUTIONS AND WARNINGS
POTENTIAL BIOHAZARDOUS MATERIAL

The reagents shall be considered a potential biohazard and handled with the same precautions applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done later. Consider all human specimens as possible biohazardous materials and take appropriate precautions to handle them.

SPECIMEN PRETREATMENT

No specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 50, and 150 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Microwell plate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater
5. Microplate washer

REAGENTS PROVIDED

1. Rabbit Anti-Estrogens Antibody Coated Break-Apart Well Microplate — Ready To Use

Contents: One polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

2. Estrogen-HRP Conjugate — Ready To Use

Contents: Estrogen-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 20 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

3. Calibrators — Ready To Use

Contents: Seven vials containing estrogen in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of estrogens.

* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 pg/mL	2.0 mL
Calibrator B	20 pg/mL	1.0 mL
Calibrator C	100 pg/mL	1.0 mL
Calibrator D	400 pg/mL	1.0 mL
Calibrator E	1000 pg/mL	1.0 mL
Calibrator F	2500 pg/mL	1.0 mL
Calibrator G	10000 pg/mL	1.0 mL

Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Controls — Ready To Use

Contents: Two vials containing estrogen in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of total estrogens. Refer to vial labels for the acceptable range.
Volume: 1.0 mL/vial
Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate — Requires Preparation X10

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. TMB Substrate — Ready To Use

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume: 16 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

7. Stopping Solution — Ready To Use

Contents: One bottle containing 1M sulfuric acid.
Volume: 6 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment: **None**.

Bring reagents, samples, and the microplate to room temperature before use. Test the calibrators, controls and specimen samples in duplicate. Once the procedure has started, complete all steps without interruption.

1. Prepare the working solution of wash buffer.
2. Remove the required number of microplate strips. Reseal the bag and return unused strips to the refrigerator.
3. Pipette 25 µL of each calibrator, control and specimen sample into planned wells in duplicate.
4. Pipette 150 µL of the Estrogen-HRP conjugate into each well. We recommend using a multichannel pipette.
5. Gently shake the plate by hand for ten seconds (or tap it on the side with your hand) to mix the contents of the wells.
6. Incubate for 2 hours at room temperature (no shaking). Cover the plate to avoid any contamination.
7. Wash the wells 3 times with 350 µL of diluted wash buffer per well. Tap the plate firmly against absorbent paper to ensure that no droplets remain in the wells. The use of a microplate washer is recommended. If a washer is not available, ensure the wash buffer reaches the top edge of the wells and no liquid remains in the plate after the final washing.)
8. Pipette 150 µL of TMB substrate into each well at timed intervals.
9. Incubate for 15 to 20 minutes at room temperature (or until calibrator A attains dark blue colour).
10. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 8. Gently tap the side of the microplate to mix the contents of the wells.
11. Read in a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Use a 4-parameter curve fit with immunoassay software to generate concentration results.
3. If no software is available draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.
4. Read the values of the unknowns off the calibrator curve.
5. If a sample reads more than 10,000 pg/mL dilute it with calibrator A at a dilution of no more than 1:10. The result obtained must be multiplied by the dilution factor.

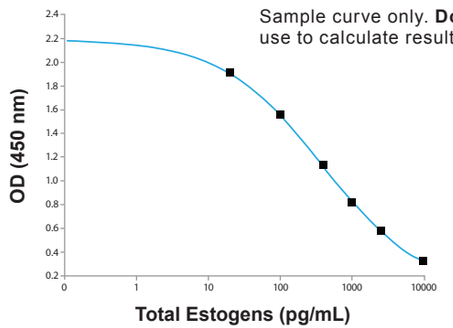
TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	OD 1	OD 2	Mean OD	Value (pg/mL)
A	2.21	2.18	2.20	0
B	1.87	1.90	1.89	20
C	1.57	1.56	1.57	100
D	1.13	1.14	1.14	400
E	0.81	0.82	0.81	1000
F	0.58	0.58	0.58	2500
G	0.33	0.33	0.33	10000
Unknown	1.00	0.96	0.98	620

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit was calculated following EP17-A. Sixty replicates of the matrix and a low concentration sample were run in independent tests with three lots of the kit. The Limit of Background was determined to be 1.9 pg/mL and the Limit of Detection was determined to be **4.4 pg/mL**.

SPECIFICITY (CROSS-REACTIVITY)

The cross-reactivity was evaluated in relation to estrogens reacting at 100%.

Steroid	% Cross-Reactivity
Estrone	100
Estradiol	100
Estriol	100
Estrone Sulfate	0.07
Estradiol Sulfate	0.15
17α-Estradiol	12.0
Equilin	10.6
DHEAS	< 0.01
Pregnenolone Sulfate	< 0.01
Cholesterol	< 0.0001

11-Hydroxycorticosterone, 17α-Hydroxyprogesterone, Aldosterone, Androstenedione, Androsterone, Corticosterone, Cortisol, DHEA, DHT, Prednisone, Pregnenolone, Progesterone, and Testosterone cross-react less than 0.1%.

The analysis of 20 patient samples from individuals on hormone replacement therapy, including patients on equilin and 17α-estradiol based drugs, yielded a correlation with LC-MS/MS of y(DBC) = 0.92x(LCMS) – 11.3, r = 0.995.

Therefore, the present device is not interfered by commonly used HRT drugs.

INTERFERENCES

Hemoglobin up to 2 g/L, Bilirubin conjugated and unconjugated up to 20 mg/dL, Triglycerides up to 5 mg/mL, Bisphenol A and Diethylstilbestrol up to 100 ng/mL, Biotin up to 10 µg/mL, Daidzein and Resveratrol up to 200 ng/mL, Genistein up to 100 ng/mL, HAMAS up to 1.2 µg/mL, and Rheumatoid Factor up to 1.2 IU/mL did not interfere with the assay.

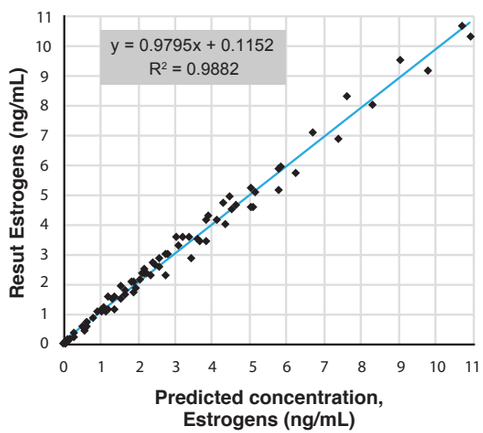
PRECISION

The experimental protocol used a nested components-of-variance design with 20 testing days, two runs per day, and two replicate measurements per run (a 20 x 2 x 2 design) for each sample. The results were analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean	Within Run SD	Within Run CV	Between Run SD	Between Run CV	Total SD	Total CV
1	60.1	6.1	10.1%	5.7	9.5%	10.9	18.2%
2	217.5	12.2	5.6%	16.8	7.7%	20.8	9.5%
3	603.1	34.6	5.7%	13.8	2.3%	41.9	7.0%
4	921.3	36.6	4.0%	44.8	4.9%	87.0	9.4%
5	1435.1	57.5	4.0%	48.3	3.4%	99.5	6.9%
6	1029	50.0	4.9%	41.3	4.0%	82.3	8.0%
7	3511.3	160.4	4.6%	122.4	3.5%	244.2	7.0%

LINEARITY

The linearity study was performed with nine human serum samples covering the range of the assay and following CLSI guideline EP6-A. The samples were diluted in calibrator A up to ten percent (1:10), tested in duplicate, and the results compared to the predicted concentration. The statistical analysis shows that the assay is sufficiently linear.



COMPARATIVE STUDIES

The DBC Total Estrogens ELISA kit (y) was compared to Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS (x). The comparison of 45 serum samples (between 20 and 4300 pg/mL) yielded the following linear regression results:
y = 1.004x - 44.3, r = 0.98

REFERENCE RANGES

Reference ranges were obtained from individuals from diverse races and without regard of menopausal status. Each laboratory shall establish their own range of reference values.

Group	N	95% Confidence Range (pg/mL)
Adult Males	120	31–167
Adult Females younger than 40 yrs.	135	36–284
Adult Females older than 60 yrs.	120	18–104

DBC-Diagnostics Biochem Canada Inc.
384 Neptune Crescent
London, Ontario, Canada N6M 1A1
Tel: (519) 681-8731
Fax: (519) 681-8734
e-mail: dbc@dbc-labs.com
www.dbc-labs.com
An ISO 13485 Registered Company

EC REP **EMERGO EUROPE**
Prinsessegracht 20
2514 AP The Hague
The Netherlands

SYMBOLS

CE European Comformity
IVD In vitro diagnostic device
Consult instructions for use
Contains sufficient for <n> tests
Storage Temperature
Legal Manufacturer
Use by
REF Catalogue Number
EC REP Authorized representative
LOT Lot number
X # Dilute 1: # Before use