Diagnostics Biochem Canada

Estrone ELISA

REF CAN-E-420

ASSAY PROCEDURE

Bring kit components to room temperature. Mix gently by inversion.

Prepare Wash Buffer Working Solution.

Pipette 50 µL of each calibrator, control and specimen sample.

Pipette 100 µL of the HRP Conjugate into each well.

Gently tap the microplate frame for 10 seconds to mix. Incubate for at room temperature (no shaking) for 60 minutes.

Wash 3 times.

Pipette 150 µL of TMB Substrate.

Incubate the microplate at room temperature (no shaking) for 20 minutes Do not tap the microplate and avoid placing in intense light or air currents.

Pipette 50 µL of Stopping Solution. Gently tap on the microplate frame to mix.

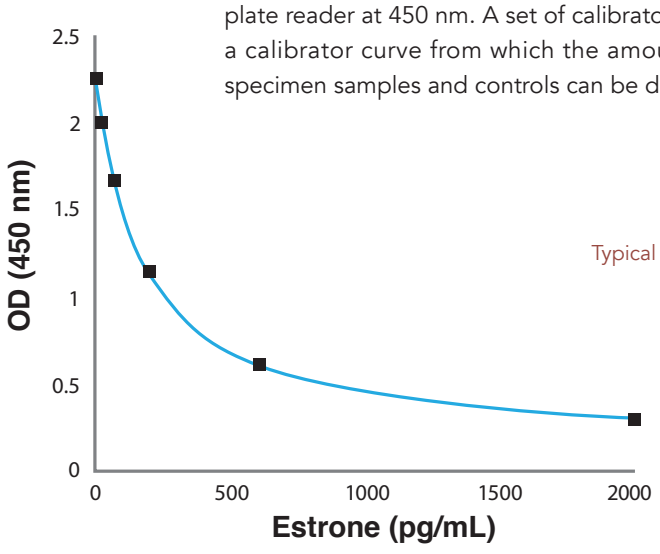
Read the plate on a microplate reader at 450 nm.

Estrone is a steroid, a female sex hormone and, with estradiol and estriol, one of the three most important endogenous estrogens. 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 in order to prepare it for the fertilized ovum.

During the follicular phase of the menstrual cycle the estrone level shows a slight increase. The production of estrone 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 and if fertilization does not occur, then the production of estrone decreases.

PRINCIPLE OF THE TEST

The Estrone ELISA is a competitive immunoassay. Competition occurs between estrone present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of anti-estrone antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-coloured product that is inversely proportional to the amount of estrone present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the colour from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of estrone in specimen samples and controls can be directly read.



Typical calibrator curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline. The Limit of Background (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) are summarized in the table below:

Parameter	Estrone (pg/mL)
LoB	5.6
LoD	14.8
LoQ	17.7

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Estrone ELISA kit with estrone cross-reacting at 100%:

Compound	% Cross-Reactivity
Estrone	100
11-Deoxycorticosterone	< 0.1
17-hydroxyprogesterone	< 0.1
17α-estradiol	3.6
17β-Estradiol	7.9
Aldosterone	< 0.1
Androstenedione	< 0.1
Androsterone	< 0.1
Cholesterol	< 0.01
Corticosterone	< 0.1
Cortisol	0.2
Danazol	< 0.1
DHEA	0.1

Compound	% Cross-Reactivity
DHEAS	< 0.1
DHT	< 0.1
Equilin	19.1
Estradiol sulfate	≤ 2.9
Estriol	2.6
Estrone sulfate	2.5
Ethisterone	< 0.1
Prednisone	< 0.1
Pregnenolone	< 0.1
Pregnenolone sulfate	< 0.1
Progesterone	< 0.1
Testosterone	< 0.1

LINEARITY

The linearity study was performed according to the CLSI EP06-Ed2 guideline using six human serum samples covering the range of the assay. The samples were diluted in low estrone value (<60 pg/mL) serum samples up to ten percent (1:10), tested in duplicate, and the regression equation of the results (y) compared to the concentration (x) predicted from the dilution factor was y = 1.001x + 10.2, r = 0.999.

The relative non-linearity ranged between -10.6% and 10.5% across all samples and measurement dilution points. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution when using low estrone value (<60 pg/mL) serum samples as the diluent.

PRECISION

The precision study was performed according to the CLSI EP05-A3 guideline.

The experimental protocol used a nested components-of-variance design with 8 serum samples, 10 testing days, two lots and two scientists per day. Each scientist ran two tests with two lots per day and two replicate measurements per run (a 10 x 2 x 2 x 2 design) for each sample. The results were analyzed with a two-way nested ANOVA and are summarized in the table below.

Sample	Mean (pg/mL)	Within Run		Between Run		Total	
		SD (pg/mL)	CV %	SD (pg/mL)	CV %	SD (pg/mL)	CV
1	91.5	8.5	9.2	11.7	12.8	14.4	15.8
2	40.7	5.1	12.4	6.2	15.1	8.0	19.6
3	144.8	11.9	8.2	15.2	10.5	20.1	13.9
4	744.4	33.4	4.5	31.7	4.3	46.7	6.3
5	632.8	26.3	4.2	41.9	6.6	56.2	8.9
6	1027.0	55.1	5.4	26.1	2.5	73.5	7.2
7	381.0	18.2	4.8	25.5	6.7	34.1	8.9
8	1211.7	53.0	4.4%	71.2	5.9%	106.2	8.8%

COMPARATIVE STUDIES

This DBC Estrone ELISA kit (y) was compared against a Liquid Chromatography-Mass Spectrometry (LC-MS/MS) method (x) and yielded the following linear regression results:

y = 0.80x + 25.82, 105 samples, r = 0.92, Slope = 0.80.

REFERENCE RANGES

Reference ranges (95%) were estimated using samples obtained from individuals of diverse races (all values are reported in pg/mL). Each laboratory shall establish their own range of reference values.

Cohort	N	Mean	Median	95% Range	
				2.5%	97.5%
Adult Female Premenopausal*	140	93.9	83.3	19.5	231.9
Adult Female, Menstrual cycle					
1–10 days	*40*	*84.4*	*81.5*	*29.8*	*146.7*
11–20 days	*40*	*87.7*	*79.6*	*20.9*	*232.0*
21–30 days	*40*	*82.2*	*73.2*	*27.2*	*173.8*
Adult Female Postmenopausal*	205	31.9	42.5	ND	166.4
Adult Male	202	59.1	52.1	ND	187.2

*The menopausal status was classified according to age.
ND = Non-Detectable; results below the LoD (14.8 pg/mL).

Ordering Information: REF CAN-E-420

DIAGNOSTICS BIOCHEM CANADA
384 NEPTUNE CRESCENT • LONDON, ONTARIO, CANADA • N6M 1A1
TEL: 519-681-8731 • FAX: 519-681-8734 • dbc@dbc-labs.com • www.dbc-labs.com