ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#**0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1926

Human Estrogens, Total

ELISA KIT Cat. No. 1926

For Quantitative Determination of Total Estrogens in Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Estrogens, Total ELISA KIT Cat. # 1926 (kit Contents)

Components	Cat. #
Rabbit Anti- Estrogens IgG Coated Microwell strip plate (96 wells)	1927
Estrogen Std. A (0 pg/ml), 2.0 ml	1928A
Estrogen Std. B (25 pg/ml), 1.0 ml	1928B
Estrogen Std. C (50 pg/ml), 1.0 ml	1928C
Estrogen Std. D (100 pg/ml), 1.0 ml	1928D
Estrogen Std. E (250 pg/ml), 1.0 ml	1928E
Estrogen Std. F (500 pg/ml), 1.0 ml	1928F
Estrogen Std. G (1000 pg/ml), 1.0 ml	1928G
Estrogen Std. H (2500 pg/ml), 1.0 ml	
Estrogen **Low & High Controls** 1.0 ml containing known values (see vial for lot sp. concn). After opening the vial, the control should be used within 14-days at 4ºC or aliquoted and stored frozen for up to 3-6 months.	1929LC, 1929HC
Estrogen-HRP conjugate, 20 ml	1930
Wash Buffer Conc. (10X), 50 ml	W 1926
HRP substrate (TMB) Solution; 16 ml	TM 1926
Stop solution, 6 ml	T - 3 0
Complete Instruction Manual	M 1926

Introduction

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.

ADI's Estrogens, Total ELISA is a rapid, specific and sensitive assay for the direct quantitative determination of Total Estrogens in human serum.

PERFORMANCE CHARACTERISTICS

PRECISION

The experimental protocol used a nested components-of-variance design with 10 testing days, two runs per scientist 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 summarized in the table below.

Sample	Mean	Within Run SD	Within Run CV%	Between Run SD	Between Run CV%	Total SD	Total CV%
1	104.6	6.6	6.3	8.3	8.0	11.9	11.4
2	56.5	5.3	9.3	7.0	12.4	8.8	15.5
3	377.2	17.6	4.7	10.8	2.9	24.4	6.5
4	83.3	4.7	5.7	4.2	5.0	7.1	8.5
5	100.2	6.0	6.0	7.5	7.4	9.9	9.9
6	251.8	10.3	4.1	13.3	5.3	17.0	6.8
7	365.9	16.8	4.6	52.2	14.3	54.8	15.0
8	1276.7	78.9	6.2	46.8	3.7	98.0	7.7

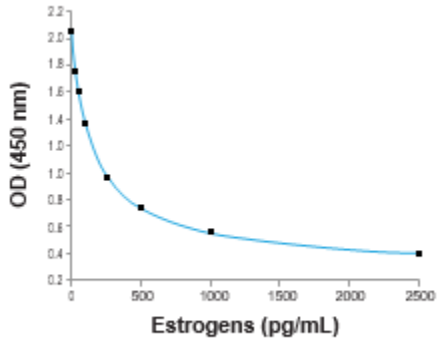
SPECIFICITY (CROSS-REACTIVITY)

Compound	% Cross-Reactivity
Estrone	100
17β-Estradiol	100
Estriol	100
11-Deoxycorticosterone	0.4
17-Hydroxyprogesterone	0.3
17α-Estradiol	5.3
Aldosterone	0.2
Androstenedione	0.2
Androsterone	0.2
Cholesterol	0
Corticosterone	< 0.01
Cortisol	< 0.1
DHEA	0.3
DHEAS	0.004
DHT	0.5
Equilin	6.3
Estradiol sulfate	0.1
Estrone sulfate	0.07
Prednisone	0
Pregnenolone	< 0.1
Pregnenolone sulfate	< 0.1
Progesterone	< 0.1
Testosterone	0.3

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (pg/ml)	Net Mean A450 nm
A1, A2	**Std. A** (0 pg/ml)	2.044
B1, B2	**Std. B** (25 pg/ml)	1.755
C1, C2	**Std. C** (50 pg/ml)	1.609
D1, D2	**Std. D** (100 pg/ml)	1.368
E1, E2	**Std. E** (250 pg/ml)	0.964
F1, F2	**Std. F** (500 pg/ml)	0.744
G1, G2	**Std. G** (1000 pg/ml)	0.561
H1, H2	**Std. H** (2500 pg/ml)	0.407

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Total estrogens ELISA kit is based upon competitive solid phase ELISA. Competition occurs between total estrogens (estrone, estradiol, and estriol) present in calibrators, controls and patient samples and an enzyme-labelled antigen (conjugate) for a limited 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 30 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 calibrator curve is plotted with a provided set of calibrators to directly calculate the concentration of total estrogens in patient samples and controls.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (10-200 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

ADI Total estrogens ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative, TMB and sulfuric acid. Necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses; therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.
TMB (substrate), H_2SO_4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Preparation of Reagents

Standards and control: All standards and controls are provided in ready-to-use solutions. Once opened, they can be kept at 4^{o}C and used within 14 days. For long-term usage, it is recommended to keep them frozen at -20^{o}C or below in suitable size aliquots. Avoid repeated freeze and thaw.

Wash buffer Concentrate (10X): Prepare 1X solution by diluting 1:10 (50 ml concentrate in 450 ml water). Store diluted stock at 4oC.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{O}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. HRP substrate should be colorless at the time of use. If solutions have turned light blue in color, these should be replaced. Do not expose these solutions to strong light during storage or use. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing are not recommended.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Prepare 1X wash buffer.**

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Save 1 well for blank and do not add anything. Pipet **50 ul of each calibrator, control, and samples** into appropriate wells.

2. Incubate the microplate on a microplate shaker for 30 minutes at room temperature.

3. Pipette 150 µL of the Estrogen-HRP conjugate into the wells containing the previously added calibrators, controls and specimen samples (the use of a multi- channel pipette is recommended).

4. Incubate the microplate on a microplate shaker for 120 minutes at room temperature.

5. Remove reaction mixture and **wash 3X with wash buffer (350 µL/well for each wash)**. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

6. Pipet **150 ul of TMB substrate Soln** into each well (blue color develops). Mix gently. Cover the plate and incubate at room temp. for **30 min** on plate shaker or until calibrator A attains dark blue color for desired OD).

7. Stop the reaction by adding **50 ul of stop** solution to all wells**.** Mix gently. Measure the absorbance at 450 nm using an ELISA reader within 15-20 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. Addition of the HRP substrate solution starts a kinetic reaction. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Do not touch the bottom of the wells.

CALCULATION OF RESULTS

1. Calculate the mean absorbance of each calibrator, control and sample duplicate and subtract the blank values.
2. Plot the concentration (X) of each reference calibrator against the mean absorbance on a semi log paper. Draw a point-to-point line through the mean of the duplicate point. If immunoassay software is used, a 4-parameter curve is recommended. Obtain the value of sample Total estrogens by standard curve.

3. If serum samples (>2500 pg/ml) dilute it with calibrator A not more than 10-fold. The result obtained must be multiplied by the dilution factor.

The results must be multiplied by dilution factor. Controls from other manufacturers may contain **serum preservatives (azide or merthiolate)** incompatible with ADI's ELISA reagents should not be used. Whenever laboratory data conflict with clinical findings or impressions, clinical judgment should be exercised, and additional evaluation undertaken. Grossly hemolyzed or lipemic samples may give erroneous results.

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	N	Median (pg/mL)	95% Reference Range (pg/mL)
Pre-menopausal Females, cycle			
1–10 days	40	120	16–328
11–20 days	40	136	34–501
21–30 days	40	168	48–350
Post-menopausal Females	120	74	40–244
Adult Males	120	104	56–213

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The Limit of Background was determined to be 5.4 pg/mL and the Limit of Detection was determined to be 12.4 pg/mL.

LINEARITY

The samples were diluted in Standard A at several equidistant concentration levels and 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 up to a 1:10 dilution when using Standard A as the diluent.

INTERFERENCES

Hemoglobin up to 2 g/L, Bilirubin conjugated and unconjugated up to 10 mg/dL, Triglycerides up to 5 mg/mL, Biotin up to 2.4 µg/mL, HAMAS up to 1.2 µg/mL, and Rheumatoid Factor up to 1500 IU/ mL did not interfere with the assay.