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-1925

ESTRONE

ELISA KIT Cat. No. 1925

For Quantitative Determination of Estrone In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

ESTRONE ELISA KIT Cat. # 1925 (kit Contents)

Components	Cat. #
Rabbit Anti-Estrone IgG Coated Microwell strip plate (96 wells)	1926
Estrone Std. A (0 pg/ml), 10 ml	1927A
Estrone Std. B (15 pg/ml), 0.5 ml	1927B
Estrone Std. C (50 pg/ml), 0.5 ml	1927C
Estrone Std. D (200 pg/ml), 0.5 ml	1927D
Estrone Std. E (800 pg/ml), 0.5 ml	1927E
Estrone Std. F (2000 pg/ml), 0.5 ml	1927F
Estrone **Low & High Controls** 0.5 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.	1927LC, 1927HC
Estrone-Biotin Conj. (100X), 200 ul	1928
Avidin-HRP conjugate. (100X), 200 ul	1929
Assay Buffer, ready to use, 15 ml	AB1925
Wash Buffer Conc. (10X), 50 ml	W1925
HRP substrate (TMB) Solution; 16 ml	TM1925
Stop solution, 6 ml	T-30
Complete Instruction Manual	M1925

Introduction

Estrone is a steroid like estriol and estradiol, belonging to the class of estrogens. 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 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.

ADI's Estrone ELISA is a rapid, specific and sensitive assay for the measurement of Estrone in human serum.

2. PRECISION

Intra-assay precision:

	Sample A	Sample B	Sample C
N	10	10	10
Mean (pg/ml)	55.40	250.50	1563.57
S.D. (pg/ml)	4.50	16.78	112.78
C.V. (%)	9.1	6.7	7.2

Inter-assay precision:

	Sample A	Sample B	Sample C
N	10	10	10
Mean (pg/ml)	55.62	260.12	1478.63
S.D. (pg/ml)	6.53	17.96	145.63
C.V. (%)	11.7	6.9	9.9

3. ACCURACY/RECOVERY

Two serum samples were spiked with known conc. of Estrone. The Estrone values were measured and % of recovery was determined.

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	52	-	-
+200	315	252	125
+400	557	452	120
+1000	1235	1052	117
2 Unspiked	75	-	-
+375	493	450	88.0
+750	505.23	559.81	90.3
+1500	712.44	794.88	89.6
3 Unspiked	720.11	-	-
+200	758.13	837.64	90.5
+400	856.46	955.17	89.7
+1000	1013.61	1190.24	85.1

3. LINEARITY

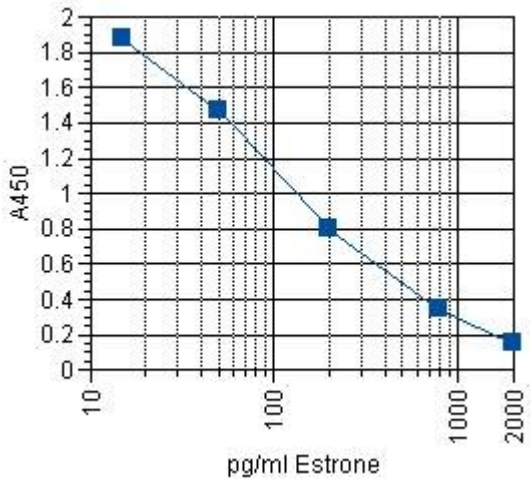
Three serum samples were diluted with Std. A. The Estrone values were measured and % of recovery was determined.

Sample	Obs.Result	Exp.Result	Recovery%
1	340.67	-	-
1:2	165.35	170.34	97.1
1:4	95.39	85.17	112.0
1:8	48.47	42.58	113.8
2	1086.01	-	-
1:2	508.58	543.00	93.7
1:4	232.11	271.50	85.5
1:8	114.95	135.75	84.7
3	1313.21	-	-
1:2	612.98	656.61	93.4
1:4	318.63	328.30	97.1
1:8	134.98	164.15	82.2

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (pg/ml)	Net Mean $A_{450\ nm}$	Calc. Conc pg/ml
A1, A2	**Std. A** (0 pg/ml)	2.15	
B1, B2	**Std. B** (15 pg/ml)	1.85	
C1, C2	**Std. C** (50 pg/ml)	1.48	
D1, D2	**Std. D** (200 pg/ml)	0.68	
E1, E2	**Std. E** (800 pg/ml)	0.35	
F1, F2	**Std. F** (2000 pg/ml)	0.21	
G1, G2	**Sample 1**	**1.32**	**65**

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

Estrone ELISA kit is based upon competitive solid phase ELISA. The patient sample competes with Streptavidin-Biotin-linked Estrone for a fixed and limited number of antibody binding sites. In the assay, the Estrone standard or samples sera are incubated with Estrone-HRP conjugate and anti-Estrone. In this solid-phase system, the antibody bound Estrone will remain on the well while unbound Estrone will be removed by washing. A color (blue) is developed when the substrate, TMB is mixed with the antibody bound Estrone-HRP conjugate. After a short incubation, the enzyme reaction is stopped (blue color turns yellow) and the intensity of the color (yellow) is measured using an ELISA plate reader. The color is inversely proportional to the concentration of Estrone in the sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (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 Estrone 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), H2SO4 (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 4oC and used within 14 days. For long-term usage, it is recommended to keep them frozen at –20oC or below is 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.

Preparation of 1X-Estrone-Biotin/Avidin-HRP Conjugate

For 10 ml of the 1X working conjugate, take 10 ml of the assay buffer and add 100 ul of estrone-biotin (#1928) and 100 ul of Avidin-HRP (#1929). Mix gently and leave it for 15-min at room temp. Add 100 ul of the prepared conjugate per well. Prepare only in the required amounts. Working dilution of the conjugate must not be stored.

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 is not recommended.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Prepare 1-X working solutions of the Estrone-biotin and Avidin-HRP conjugate. It must be prepared and left at room temp for 15-min prior to its addition to the plates. Prepare 1X wash buffer.**

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 ul of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Save 1 well for blank and do not add anything. Pipet **50 ul stds. and samples** into appropriate wells.

2. Pipet **100 ul of working solution of estrone-biotin-avidin conjugate** solution into each well except blank well. Mix gently for 5-10 seonds. Cover the plate and incubate at room temp. for **60 min** on plate shaker (approx 200 rpm).

3. Remove reaction mixture and **wash 3X with wash buffer**. 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.

4. Pipet **150 ul of TMB substrate Soln** into each well (blue color develops). Mix gently. Cover the plate and incubate at room temp. for **15 min** on plate shaker or until calibrator A attains dark blue color for desired OD).

5. 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 standards and duplicate and subtract the blank values.
2. Plot the concentration (X) of each reference standard 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 Estrone by standard curve.
3. If serum samples (>2000 pg/ml) were diluted then the values must be multiplied by the dilution factor.

DILUTION OF SAMPLES and LIMITATIONS

It is recommended that each laboratory must determine its own normal and abnormal ranges. Extrapolation of Estrone values beyond the standard curve may yield variable results. Samples containing >2000 pg/ml Estrone can be diluted with 0 standard (no more than 1:8 dilution) and retested. 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	Range (pg/ml)
Males	25-150
Females	25-350
Pregnancy	100-8000

PERFORMANCE CHARACTERISTICS

SPECIFICITY (CROSS REACTIVITY)

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

Steroid	%Cross Reactivity
Estrone	100
Estrone-3-Sulfate	4.9
17β-Estradiol	2.2
Estrone-3-Glucuronide	1.2
17ß-Estradiol-3-Glucuronide	0.14

The following steroids were tested but cross-reacted at less than 0.1%: Androstenedione, Cholesterol, Corticosterone, Cortisol, Cortisone, DHEAS, Diethylstilbesterone, Estriol, 17ß-Estradiol-3-Glucuronide, Estradiol-Sulfate, Progesterone, 17-OH Progesterone and Testosterone

Sensitivity

The minimal detectable conc. of Estrone is estimated to be **10 pg/ml**. The minimal detectable conc. is defines as the concn. of Estrone, which corresponds to the absorbance, that is 2 S.D. smaller than the mean abs. Value of the zero std.