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		

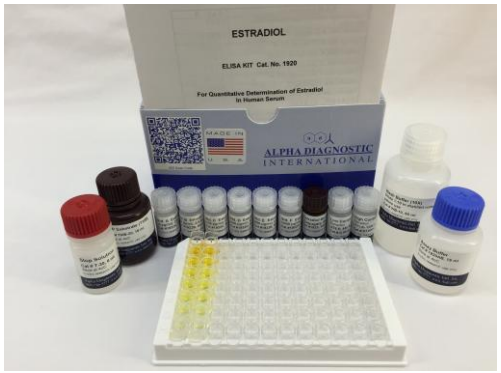
#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1920

ESTRADIOL

ELISA KIT Cat. No. 1920

For Quantitative Determination of Estradiol In Human Serum



For In Vitro Research Use Only (RUO)

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ESTRADIOL ELISA KIT Cat. No. 1920

Kit Contents: (reagents for 96 tests)

Components	Cat. #
Anti-Estradiol coated microwell strip plate (96 wells)	1921
Estradiol **Std. A** (0 pg/ml), 2 ml	1922A
Estradiol **Std. B** (20 pg/ml), 0.5 ml	1922B
Estradiol **Std. C** (100 pg/ml), 0.5 ml	1922C
Estradiol **Std. D** (300 pg/ml), 0.5 ml	1922D
Estradiol **Std. E** (800 pg/ml), 0.5 ml	1922E
Estradiol **Std. F** (3200 pg/ml), 0.5 ml	1922F
Estradiol **Low & High Controls** (see vials for lot sp. conc.) C1920H, C1920L	
Standards and controls are provided in protein-based stabilized buffer containing known concn of Estradiol. Lot specific exact concn are provided on the vial. The standards can be kept at 4oC when unused and unopened. After the first usage, the standards should be kept at -20oC or below in suitable size aliquots. Avoid repeated freeze and thaw.	
Estradiol HRP Conjugate (50X); 0.3 ml, (before use dilute 1:50 with assay buffer)	1923
Wash Buffer Conc. (10X), 50 ml	WB-10
Assay Buffer, 15 ml	1920AB
HRP substrate (**TMB**) Solution; 16 ml	TMB-20
Stop solution, 6 ml	T-30
Complete Instruction Manual	M1920

Introduction

Estradiol is one of the main component of naturally occurring estrogens and is the major estrogen secreted during the menstrual cycle. The serum levels of Estradiol are low during the follicular phase rising gradually until about one day before the ovulation when a marked rise in the Estradiol level occurs (ovulatory peak). The Estradiol level fall rapidly at, or right after ovulation and is again within the levels of the follicular phase. There is a second rise of Estradiol around day 21 of the cycle (luteal phase). The levels then decline gradually to the lowest level at the onset of the next menstrual cycle.

ADI's Estradiol ELISA kit provides for the measurement of Estradiol in human serum.

2. PRECISION

Intra-assay precision:

	Sample A	Sample B
N	7	7
Mean (pg/ml)	192.76	465.08
S.D.(pg/ml)	14.54	56.72
C.V. (%)	7.54	12.2

Inter-assay precision:

	Sample A	Sample B
N	8	8
Mean (pg/ml)	65.49	555.01
S.D.(pg/ml)	7.89	45.27
C.V. (%)	12.05	8.16

3. ACCURACY/RECOVERY

Two serum samples were spiked with standard E, F, and G (1:1). The Estradiol values were measured and % of recovery was determined.

Initial Values (pg/ml)	Observed values (pg/ml)	Expected values (pg/ml)	Recovery (%)
Sample A Unspiked	24.0		
+ Std E	278	212	113
+ Std. F	421	412	102
+ std. G	883	1012	87
Sample B Unspiked	654.0		
+ Std E	1128	1027	110
+ Std. F	1378	1227	112
+ std. G	1815	1827	99

3. LINEARITY

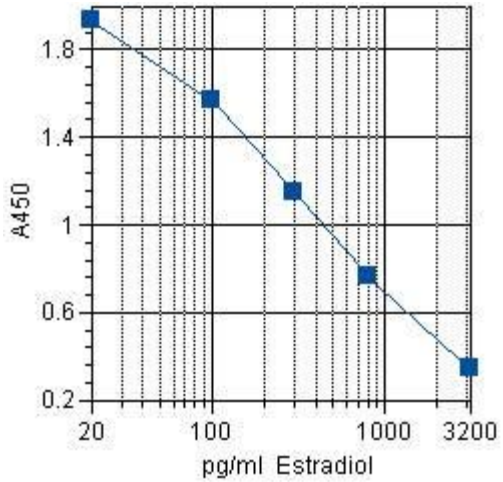
Two serum samples were diluted with Std. A. The Estradiol values were measured and % of recovery was determined.

Initial Values (pg/ml)	Observed values (pg/ml)	Expected values (pg/ml)	Recovery (%)
Sample A Undiluted	403		
Dilution 1:2	210.5	202.0	104.0
Dilution 1:4	86.8	101.0	86.0
Dilution 1:8	39.2	51.0	77.0
Sample B Undiluted	668		
Dilution 1:2	276	334	83.0
Dilution 1:4	149.0	167.0	89.0
Dilution 1:8	78.0	84.0	93.0

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Net Mean $A_{450\ nm}$
A1, A2	**Std. A** (0 pg/ml)	2.088
B1, B2	**Std. B** (20 pg/ml)	1.935
C1, C2	**Std. C** (100 pg/ml)	1.575
D1, D2	**Std. D** (300 pg/ml)	1.157
E1, E2	**Std. E** (800 pg/ml)	0.759
F1, F2	**Std. F** (3200 pg/ml)	0.337

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)

CALCULATION OF RESULTS

1. Calculate the net mean OD from the duplicates of standards, controls, and patients samples.
2. Plot the concentration (X) of each reference standard against its Absorbance (Y) using a semi-log paper. Draw a point-to-point line through the mean of the duplicate point.

Obtain the value of sample Estradiol by standard curve. The data given in the example is for demonstration purpose only and must not be used in place of data for each assay.

PRINCIPLE OF THE TEST

Estradiol ELISA kit is based upon competitive solid phase ELISA. The patient sample competes with enzyme-linked Estradiol for a fixed and limited number of antibody binding sites on the coated plates. In the assay, the Estradiol standard or samples sera are incubated with Estradiol-HRP conjugate in the anti-Estradiol coated wells. In this solid-phase system, the antibody bound Estradiol will remain on the well while unbound Estradiol will be removed by washing. A color (blue) is developed when the substrate, TMB is mixed with the antibody bound Estradiol-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 Estradiol in the sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Estradiol ELISA test is intended for *in vitro research* use only. The reagents contain proclin-300 as preservative; 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.

REAGENT PREPARATION

1. **Prepare 1x wash buffer** by diluting 10X stock (**50 ml stock into 450 wate**r).

2. **Prepare 1X Estadiol- HRP Conjugate (#1923)**

Dilute stock conjugate 1;50 in assay buffer (20 ul stock conjugate in 1 ml of assay buffer; prepare 11 ml for a full plate). Prepare 1x conjugate in required volume and do not store diluted conjugate for more than a few hours. All items must be at room temp prior to dispensing into the plate.

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 working solution of 1X Conjugate (see page 2). Prepare 1X wash buffer (page 2).***

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

2. Pipet **50 ul of standards,** control, and serum samples into appropriate wells in *duplicate*.

3. Add **100 ul of 1X Estradiol-HRP** conjugate into each well**.** Mix gently for 5-10 seconds. Cover the plate and incubate **on a plate shaker** for **60 minutes** at room temperature.

4. 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.

5. Pipette **150 ul of TMB HRP-substrate solution** into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for **10-15 minutes** at room temperature **on a plate shaker** (blue color develops in standards and samples). The incubation step can be controlled to give the Standard A reading A450 of 2.0.

6. Stop the reaction by adding **50 ul of stop solution** to all wells**.** Mix gently for 5-10 seconds (blue color turns yellow). Measure the absorbance at 450 nm using an ELISA reader within 30 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. The unused strips should be stored in a sealed bag at 4^{O}C. 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.

DILUTION OF SAMPLES and LIMITATIONS

It is recommended that each laboratory must determine its own normal and abnormal ranges. Extrapolation of Estradiol values beyond the standard curve may yield variable results. Samples containing >3200 pg/ml Estradiol 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 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.

INTERPRETATION EXPECTED VALUE

1. It is recommended that each laboratory should determine its own normal and abnormal range. The following values can be used as preliminary guidelines until the laboratory establishes its own normal values.

Male	15-100 pg/ml
Female	15-400 pg/ml
Postmenopausal	15-90 pg/ml

Specificity

The estradiol antibody used in this kit is very sensitive and specific for Estradiol. The following compounds were tested for crossreactivity of the assay: Estradiol (100%), Estriol and Estrone (1%), Progesterone, and Cortisol (0.1%).

Sensitivity

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

Species Crossreactivity

This kit has been designed and tested for human serum samples. It may be optimized for other human biological fluids. It has not been tested in animals (rat, mouse, etc). Since the steroid hormone is the same in all species, this kit should work in most species as long as the sample cocn is within the range of this kit. This kit has been used in mouse and rat samples.

List of Publications using ADI' Estradiol ELISA Kit #1920

Mankhey RW, 2006, Am J Physiol Regulatory Integrative Comp Physiol, in press, used for **rat plasma Estradiol** using ELISA

Yu A-M, 2005, Endocrinol., 146: 2911 – 2919, **Human Estradiol**

Yang Q, 2006, Endocrinology, 147: 4772–4780, **mouse serum** Estradiol