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-1935

Free Estriol ELISA

ELISA KIT Cat. No. 1935

For the quantitative determination of free estriol in human serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Free **Estriol ELISA KIT Cat. No. 1935**

For Quantitative Determination of free estriol in human serum.
Kit Contents: (reagents for 96 tests)

C o m p o n e n t s	
Anti-estriol coated microwell strip plate (96 wells), Ready-to-use #1 9 3 6	1 P l a t e
Free Estriol **Standard A**,1 ml, #1 9 3 7 A	1 V i a l
Free Estriol **Standard B,** 1 ml, #1 9 3 7 B	1 V i a l
Free Estriol **Standard C,** 1 ml , #1 9 3 7 C	1 V i a l
Free Estriol **Standard D,** 1 ml, #1 9 3 7 D	1 V i a l
Free Estriol **Standard E,** 1 ml, #1 9 3 7 E	1 V i a l
Free Estriol **Standard F,** 1 ml, #1 9 3 7 F	1 V i a l
Free Estriol **Low Control**, 1 ml, #1 9 3 5 L C	1 V i a l
Free Estriol **High Control**, 1 ml, #1 9 3 5 H C	1 V i a l
HRP **Conjugate** ; 14 ml , #1934	1 b o t t l e
HRP **substrate Solution**; 14 ml, #TMB-1935	1 b o t t l e
Stop solution, 14 ml, #ST-1935	1 b o t t l e
Wash Buffer (40X), 30 ml, #WB-1935	1 b o t t l e
Complete Instruction Manual, M - 1 9 3 5	1

Introduction

Estriol (E3) is the major estrogen formed by the fetoplacental unit during pregnancy. Unconjugated E3 passes into the placenta into the maternal circulation, where it is rapidly converted into glucuronide and sulfate derivatives to facilitate its excretion. The half-life of estriol in the maternal bloodstream is only 20-30 minutes. Its measurement therefore offers a convenient and quick evaluation of current fetal status. Plasma estriol levels increase steadily throughout pregnancy and most rapidly during the third trimester (28-40) weeks. A sudden decrease in fetoplacental E3 production will result in a rapid fall in unconjugated E3 in the maternal serum. There are several potential advantages to measuring unconjugated E3 rather than total serum or urinary E3. Unconjugated estriol levels are free from effects related to maternal renal or hepatic disease and are not altered by the administration of certain antibiotics. Unconjugated E3 more accurately reflects fetal outcome in diabetic pregnancies- and since no hydrolysis of unconjugated E3 is required, a more rapid turnaround for the test result is possible.

The ADI's E3 EIA provides a direct non-isotopic method for measuring unconjugated estriol in unextracted human serum. This method uses a highly specific estriol antibody and an enzyme- labeled analyte. The colored-end product may be measured on a spectrophotometer. The long shelf life of this product, together with the elimination of radioisotopes, radiation counter and necessary licensing requirements make this method applicable to both large and small laboratories.

PERFORMANCE CHARACTERISTICS

Assay Dynamic Range:
The range of the assay is between 0.075 – 40 ng/mL.

Specificity of Antibodies (Cross Reactivity)
The following substances were tested for cross reactivity of the assay:

Added steroid	Conc.of steroid	OD 450	Measured conc.
Estriol (E3)	40 ng/mL	**0.39**	39.67 ng/mL
Testosterone	16 ng/mL	**1.758**	n.d.
Estradiol	2 ng/mL	**1.579**	n.d.
Estrone	2 ng/mL	**1.712**	n.d.
Cortisol	800 ng/mL	**1.775**	n.d.

n.d. = non detectable

Sensitivity:

The analytical sensitivity of the ADI's ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Standard 0 and was found to be 0.075 ng/mL.

Intra-Assay:
The within-assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	20	2.1	4.7
2	20	6.2	3.2
3	20	14.6	3.0

Inter-Assay:
The between-assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	12	2.1	4.6
2	12	5.7	8.5
3	12	13.3	9.5

Recovery:

Recovery of ADI's ELISA was determined by adding increasing amounts of the analyte to three different patient sera containing different amounts of endogenous analyte. Each sample (nonspiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

	Sample 1	Sample 2	Sample 3
Conc. (ng/mL)	1.3	3.6	7.8
Average Recovery	100.8	101.8	106.9
Range of Recovery %. from	89.0	92.3	98.5
Range of Recovery % to	103.8	109.8	112.3

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Net Mean $A_{450\ nm}$
A1, A2	**Std. A** (0)	1.79
B1, B2	**Std. B** (0.3)	1.48
C1, C2	**Std. C** (1.2)	1.18
D1, D2	**Std. D** (4.0)	0.81
E1, E2	**Std. E** (15.0)	0.52
F1, F2	**Std. F** (40.0)	0.38

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.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method The results in the Instructions for Use have been calculated automatically using a 4 Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as
> 40 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

EXPECTED NORMAL VALUES:

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Normal healthy adults:

In a study conducted with apparently normal healthy adults, using the ADI's Estriol free ELISA the following values are observed:

Population	Valid N	5% Percentile [ng/mL]	5% Percentile [ng/mL]
Males	42	0.146	0.573
Females(Not pregnant)	43	0.057	0.822

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

PRINCIPLE OF THE TEST

Free Estriol (antigen) in the sample competes with the antigenic estriol conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti estriol coated on the microplate (solid phase). After incubation, the bound/free separation is performed by a simple solid-phase washing. Then the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue color that changes into yellow when the Stop Solution is added. The color intensity is inversely proportional to the Total Estriol concentration in the sample. Total Estriol concentration in the sample is calculated based on a series of standards.

MATERIALS AND EQUIPMENT REQUIRED

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

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), Hcl (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

PRECAUTIONS

The Alpha Diagnostic International ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal 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.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating 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

Wash buffer (40X): add deionized water to the 40X concentrated Wash Solution. Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL. *The diluted Wash Solution is stable for 2 weeks at room temperature.*

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. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

Specimen Dilution:

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Test Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

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

1. Pipet **10 ul of standards**, control, and serum samples into appropriate wells in *duplicate*.

2. Add 1**00 ul of 1X HRP** conjugate into **each well.** Mix gently and incubate for **60 min** at **37oC**.

3. Remove incubation mixture and **wash the wells 4X** with 400 ul wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Dispense **100 ul TMB substrate per well.** Mix the plate gently for 5-10 seconds. Cover the plate and incubate at **room temp** (25-28oC) in the dark, for **30 minutes.** Blue color develops in standards and positive wells.

5. Stop the reaction by adding **100 ul of stop solution** to **all wells.** Mix gently for 5-10 seconds (blue color turns yellow). Measure the **A450 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 4oC.

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. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

LIMITATIONS OF USE:

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

Interfering Substances

Haemoglobin (up to 4 mg/mL), bilirubin (up to 0.125 mg/mL) and triglyceride (up to 30 mg/mL) have no influence on the assay results.

Drug Interferences:

Until today no substances (drugs) are known to us, which have an influence to the measurement of Estriol in a sample.

High-Dose-Hook Effect

No hook effect was observed in this test.

General References:

Bashore, R.A., Westlake, J.R. Plasma unconjugated estriol values in high risk pregnancy.
Am. J. Obstet. Gynecol., June 15, 1977, p371-380