



## **Estriol (Free E3) RIA**

For the quantitative determination of unconjugated estriol in human serum or plasma

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	38-FE3HU-R120
Size:	120 tests
Version:	10-05 - ALPCO 2/17/2010

### **ALPCO Diagnostics**

*26G Keewaydin Drive • Salem, NH 03079*  
*Phone: (800) 592-5726 • Fax: (603) 898-6854*  
*[www.alpco.com](http://www.alpco.com) • Email: [web@alpco.com](mailto:web@alpco.com)*

## 1. INTENDED USE

The kit allows for the quantitative determination of unconjugated estriol by RIA in human serum or plasma samples over the range of 0.5 to 40 ng/ml. This kit is not to be used for the risk evaluation of trisomy 21. This kit is for research use only. It is not for use in diagnostic procedures.

## 2. SUMMARY AND EXPLANATION OF THE TEST

Estriol (1,3,5(10)-Estratriene-3,16 $\alpha$ ,17 $\beta$ -triol) is a C18 steroid hormone with a molecular weight of 288.4 daltons. It is the principal circulatory estrogen hormone in the blood during pregnancy. During pregnancy, estriol production is predominantly from the fetal/placental unit (1). It is released from the placenta and conjugated in the maternal liver as glucuronides and sulfates. It exists in the maternal blood unconjugated (8%) and in conjugated forms (92%). Maternal serum unconjugated estriol levels increase rapidly following the first trimester from 1.2 ng/ml at 15 weeks to about 12 ng/ml at term. Estriol circulating in the blood has a short half-life of 20-30 minutes (2, 3), and so variation in the fetal/placental unit should rapidly be reflected by changes in the maternal serum estriol levels. Measurement of serum unconjugated estriol can therefore be useful to monitor fetal conditions (4). Consistently low levels of estriol throughout pregnancy, or a sudden drop of estriol levels in serial determination may be indicative of fetal distress (5) or placental failure. Chronically low estriol values may be caused by other factors unrelated to fetal distress or placental failure (6).

## 3. PRINCIPLE OF THE PROCEDURE

The proposed method allows the quantitative determination of unconjugated estriol by RIA in serum or plasma samples, without preliminary treatment of the sample.

The antigen, whether from standards or samples, competes with the radioactive tracer ( $I^{125}$  estriol) for the binding sites of the antibody. After incubation the antigen-antibody complex is separated from unbound antigen by the addition of the second antibody coupled to magnetic particles. The application of a magnetic field allows precipitation of the bound fraction, eliminating the need for centrifugation.

After decantation the radioactivity is read by gamma-counter. The unknown values are obtained from the standard curve by interpolation.

## 4. REAGENTS- Preparation and Storage

Do not interchange reagents from different kit lots. Lot numbers of reagents should be stated on the "Certificate of Analysis". Do not use kit components beyond their expiry date.

The Estriol (Free E3) RIA kit (38-FE3HU-R120) contains sufficient reagents for 120 tubes. On receipt, store the kit at 2-8°C until the expiry date on the kit label.

- 1)  **$I^{125}$  Tracer, 1 vial (Red):** Contains estriol labeled with  $I^{125}$  in buffer solution with dyes and preservatives. 12.5 ml per vial. Maximum Radioactivity: 120 kBq. The reagent is ready to use. Allow the vial to equilibrate to room temperature (18-25°C) and mix thoroughly by gentle inversion, avoiding foam before use. Store at 2-8°C until the expiry date printed on the vial label.
- 2) **Calibrators, 6 vials:** Concentrations of estriol in the human serum base are: 0, 0.5, 1.5, 4.0, 10.0, and 40.0 ng/ml. 1.0 ml per vial for the Zero Calibrator, 0.5 ml per vial for Calibrators 1-5. The solutions are ready for use. Store at 2-8°C until the expiry date printed on the vial label.

- 3) **Separation Reagent, 1 vial:** Contains anti-rabbit gamma globulins (raised in goat) and coupled to magnetic particles and preservatives. 120 ml per vial. The suspension is ready for use. Store at 2-8°C until the expiry date printed on the vial label. Do not freeze.
- 4) **Antiserum, 1 vial:** Contains anti-estriol antiserum (raised in rabbit) in phosphate buffer and BSA, with dyes and preservatives. 12 ml per vial. The solution is ready for use. Store at 2-8°C until the expiry date printed on the vial label.
- 5) **Serotest S, 1 vial:** Reconstitute the contents of the vial with 1 ml deionized water and mix until the freeze-dried residue is completely dissolved. Store at 2-8°C for 3 days or at -20°C for longer periods of time.

**Note:** Appearance of moisture in the lyophilized Serotest S may indicate reagent deterioration.

## 5. WARNINGS AND PRECAUTIONS FOR USERS

*This kit is for research use only. It is not for use in diagnostic procedures.*

*Only experienced laboratory personnel should use this test and handling should be in agreement with GLP.*

*Radioactive Material – Not for Internal or External Use in Humans or Animals.*

This radioactive material may be received, acquired, possessed, and used only by physicians, research laboratories or hospitals and only for laboratory tests not involving internal or external administration of the material, or the radiation there from to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations of each country.

Physical characteristics of I<sup>125</sup>:

$$t_{1/2} = 59.9 \text{ days}$$

	E (MeV)	%
$\gamma$	0.035	
X	0.027	114
	0.032	25

1) **SAFETY PRECAUTIONS:** The following precautions should be observed when handling radioactive material:

- Store radioactive materials in a designated area.
- Do not eat, drink, smoke, or apply cosmetics where radioactive materials are being handled.
- Do not pipette by mouth.
- Wear gloves when handling radioactive materials and wash hands thoroughly afterwards.
- Cover working area with disposable absorbent paper.
- Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste.
- Dispose of the liquid radioactive waste into the sanitary sewage system if permitted by the local regulations.

2) **CHEMICAL HAZARDS Sodium Azide (NaN<sub>3</sub>) Warning:** Some of the reagents in this kit contain sodium azide as a preservative. For all such reagents, the concentration of sodium azide is <0.1% p/p. Sodium azide may react with lead and copper plumbing to form explosive metal azides. When disposing of non-radioactive reagents through the plumbing system flush with large amounts of water.

### Risk Phrases

**R 21/22** Harmful in contact with skin and if swallowed.

## Safety Phrases

**S-26** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

**S-28.1** After contact with skin, wash immediately with plenty of water.

**S-46** If swallowed, seek medical advice immediately and have the container or label available.

3) **POTENTIALLY BIOHAZARDOUS MATERIAL Warning:** This kit may contain some reagents made with human serum or plasma. The serum or plasma used has been tested by an FDA-approved method and found to be non-reactive for HIV-1/2 Antibodies, HCV, and HBsAg. Because no method can offer complete assurance that HIV-1/2, HCV, HBsAg, or other infectious agents are absent, these reagents should be handled at Biosafety Level 2 as recommended for any potentially infectious human serum or blood sample in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 3<sup>rd</sup> Edition 1993.

## 6. SAMPLE COLLECTION AND STORAGE

Serum or plasma can be used.

**Plasma:** add an anticoagulant (EDTA or Heparin), centrifuge for 10 minutes and collect the plasma.

**Serum:** allow the blood to clot at room temperature. Centrifuge for 10 minutes and collect the serum.

**Storage:** If the assay is performed within 24 hours from collection, store at 2-8°C; for longer periods of time, store the samples at -20°C. Avoid thawing the sample more than once; if necessary, aliquot the sample.

If the sample is turbid after thawing, it is recommended to centrifuge the sample before the assay. Grossly lipemic or badly hemolyzed samples must not be used.

## 7. ASSAY PROCEDURE

### 1) Materials Provided

The Estriol (Free E3) RIA is sufficient for 120 tubes and contains the following reagents:

Reagents	Quantity
I <sup>125</sup> Tracer	1
Calibrators	6
Antiserum	1
Separation Reagent	1
Serotest S	1

### 2) Materials and Equipment Required but not Provided

- Deionized water for Serotest S reconstitution
- Plastic test tubes (~1 x 7 cm)
- Automatic micropipettes with disposable tips (0.02 - 0.1 ml)
- Automatic dispenser for addition of Separation Reagent
- Vortex
- Rack: 60 tubes rack for use in Magnetic Separator
- Magnetic Separator: magnetic plate to be used for Bound/Free separation by double antibody coupled to magnetic particles
- Gamma-counter (efficiency >70% is recommended)
- Centrifuge

### 3) Preparation for Assay

Allow the reagents to equilibrate to room temperature and mix gently before use.

For each assay, prepare 5 groups of tubes in the Rack:

- 2 tubes for total radioactivity
  - 2 tubes for NSB (non-specific binding, no antiserum added)
  - 2 tubes for B<sub>0</sub> ("0" concentration of cold antigen)
  - 2 tubes for each Calibrator
  - 2 tubes for each sample and Serotest S
- 
- a) Pipette 0.02 ml of each Calibrator, Serotest S, and sample into the respective tubes. It is important to dispense them into the bottom of the tubes.
  - b) Pipette 0.02 ml of the Zero Calibrator into the NSB and B<sub>0</sub> tubes.
  - c) Add 0.1 ml of deionized water to the NSB tubes.
  - d) Pipette 0.1 ml of the Tracer into every tube - a red color will be seen in the tubes.
  - e) Mix the tubes on the vortex.
  - f) Pipette 0.1 ml of the Antiserum into all tubes except NSB and total radioactivity tubes – a dark green color will be seen in the tubes.
  - g) Mix on the vortex and incubate for 1 hour at room temperature (18-25°C).
  - h) Add 1 ml of the Separation Reagent to all tubes except the total radioactivity tubes. While dispensing, the Separation Reagent vial should be occasionally swirled to ensure uniformity.  
**ATTENTION: do not use magnetic stirrer to homogenize the suspension.**
  - i) Mix all the tubes on the vortex and incubate for 10 minutes at room temperature. Remove the total radioactivity tubes from the rack.
  - j) Slide the Rack into the Magnetic Separator and ensure that the tubes are in contact with the surface of the separator; wait for 10 minutes.
  - k) Decant the supernatant from the tubes by inversion of the Magnetic Separator. Place the inverted Magnetic Separator on absorbent paper in order to remove the drops of liquid adhering to the walls of the tubes.
  - l) Count all tubes, including the total radioactivity tubes, for at least 1 minute in the gamma-counter.

#### 4) Assay Procedure Scheme

The volumes are expressed in ml. It is recommended to mix the reagents before use.

Reagents	Tubes				
	Sample	B <sub>0</sub>	Calibrators 1-5	NSB	Total Radioactivity
Sample or Serotest S	0.02	-	-	-	-
Zero Calibrator	-	0.02	-	0.02	-
Deionized water	-	-	-	0.1	-
Calibrators 1-5	-	-	0.02	-	-
I <sup>125</sup> Tracer	0.1	0.1	0.1	0.1	0.1
Mix on vortex					
Antiserum	0.1	0.1	0.1	-	-
Mix on vortex and incubate for 1 hour at room temperature (18-25°C)					
Separation Reagent	1.0	1.0	1.0	1.0	-
Mix on vortex and incubate for 10 minutes at room temperature					
(Remove total radioactivity tubes from the Rack) Place the Rack in the Magnetic Separator and wait for 10 minutes					-
Decant the supernatant					-
Count in the gamma-counter for 1 minute					

#### 5) Conversion Factor

Conversion to nmol/l concentration units: 1 nmol/l = 1 ng/ml x 3.47.

#### 6) Quality Control

Values for Serotest S (control serum) and other control sera should fall within the confidence ranges established in each laboratory. Control ranges are printed on the "Certificate of Analysis" which is supplied with each kit.

### 8. CALCULATIONS AND RESULTS

NSB mean counts may be subtracted from all mean counts except from the total radioactivity counts. B<sub>0</sub> mean counts are used to calculate the % binding in absence of cold antigen (maximum binding).

B<sub>0</sub> mean counts

-----  
Total radioactivity mean counts x 100 = % binding

% relative binding of Calibrators and samples is calculated with the following formula:

Mean counts (Calibrator, sample, or Serotest S)

-----  
B<sub>0</sub> mean counts x 100 = % relative binding

Draw the dose-response curve by plotting the % relative binding of each Calibrator (y axis) against the relative concentration (x axis) using semilogarithmic paper (or appropriate computer program). Interpolate the % relative binding of each sample on the standard curve to obtain the sample antigen concentration in ng/ml. For diluted samples it is necessary to multiply the value read on the standard curve by the dilution factor.

**Example of Calculation - Representative Standard Curve**

<b>Tubes</b>	<b>Mean cpm</b>	<b>B/B<sub>0</sub> (%)</b>	<b>Concentration (ng/ml)</b>
Total radioactivity	25029	-	-
NSB	657	-	-
B <sub>0</sub>	22138	-	-
Calibrator 1 - 0.5 ng/ml	20492	92.33	-
Calibrator 2 - 1.5 ng/ml	17799	79.80	-
Calibrator 3 - 4.0 ng/ml	12856	56.79	-
Calibrator 4 - 10.0 ng/ml	9215	39.84	-
Calibrator 5 - 40.0 ng/ml	4916	20.04	-
Sample 1	17448	78.17	1.49

**9. LIMITATIONS**

- 1) Follow the instructions in this package insert carefully to obtain reliable results.
- 2) Do not use the reagents beyond the expiry date stated on the vial labels.
- 3) Do not mix reagents from different lots.
- 4) It is recommended to follow the incubation times in the package insert to avoid variation of the analytical results.
- 5) Samples contaminated with endogenous radioactivity may give inaccurate results.

**10. EXPECTED VALUES**

<b>Pregnancy week</b>	<b>Median (ng/ml)</b>	<b>Range 90<sup>th</sup> percentile (ng/ml)</b>
25 <sup>th</sup>	3.25	1.86 – 6.2
26 <sup>th</sup>	3.9	2.23 - 5.61
27 <sup>th</sup>	4.37	3.15 - 6.15
28 <sup>th</sup>	4.49	2.26 - 5.4
29 <sup>th</sup>	3.55	2.28 - 6.7
30 <sup>th</sup>	3.8	2.24 - 7.7
31 <sup>st</sup>	4.66	3.06 - 7.86
32 <sup>nd</sup>	4.92	3.6 - 7.49
33 <sup>rd</sup>	6.34	4.82 - 13.1
34 <sup>th</sup>	5.77	3.32 - 17.0
35 <sup>th</sup>	6.7	4.08 - 13.15
36 <sup>th</sup>	8.1	4.96 - 15.8
37 <sup>th</sup>	9.39	6.29 - 18.76
38 <sup>th</sup>	10.62	6.47 - 20.51
39 <sup>th</sup>	11.37	9.9 - 20.33
40 <sup>th</sup>	10.95	5.73 - 20.41

It is recommended that each laboratory establishes its own normal range.

## 11. PERFORMANCE CHARACTERISTICS

### 1) Accuracy

Recovery studies were performed by adding free estriol to pooled serum samples. Free estriol values were determined before and after addition, and the % recovery of added Free estriol calculated.

Sample	Free Estriol Added (ng/ml)	Free Estriol Measured (ng/ml)	Recovery (%)
1	0	0	-
	0.49	0.50	102.0
	0.98	1.03	105.1
	1.96	2.14	109.2
	3.92	3.76	95.9
	7.84	7.35	93.8
	15.68	14.11	90.0
	31.35	28.88	92.1

### 2) Dilution Test

Dilution was examined by diluting serum samples with the Zero Calibrator. Results are shown below:

Sample	Dilution Factor	Free Estriol Measured (A) (ng/ml)	Free Estriol Theoretical (B) (ng/ml)	A/B%
1	-	25.40	25.40	-
	2	12.10	12.70	95.3
	4	6.35	6.35	100.0
	8	3.48	3.18	109.4
	16	1.66	1.59	104.4
	32	0.86	0.79	108.9
2	-	29.30	29.30	-
	2	13.40	14.65	91.5
	4	7.0	7.33	95.5
	8	3.86	3.66	105.5
	16	1.96	1.83	107.1
	32	0.94	0.92	102.2
3	-	31.94	31.94	-
	2	15.53	15.97	97.2
	4	7.55	7.99	94.5
	8	4.17	3.99	104.5
	16	2.03	2.00	101.5
	32	1.01	1.00	101.0

### 3) Precision

#### Intra-assay

3 serum pools were measured 15 times in the same assay. The within assay variability is shown below:

Sample	Mean (ng/ml)	SD	CV%
A	1.04	0.04	4.1
B	4.57	0.18	3.8
C	9.12	0.24	2.6

#### Inter-assay

3 serum pools were measured in 10 different runs. The between-assay variability is shown below:

Sample	Mean (ng/ml)	SD	CV%
A	1.09	0.08	11.9
B	4.51	0.21	6.8
C	8.99	0.43	4.9

#### 4) Sensitivity

The sensitivity of the method, defined as the concentration of free estriol equivalent to the mean cpm of 20 replicates of the Zero Calibrator minus 2 standard deviations, is typically 0.25 ng/ml.

#### 5) Specificity

The specificity has been evaluated by the interference of the following steroid compounds, according to Abraham's method ( $x/y \times 100$ ) where x and y are respectively the weight of free estriol and of the interfering compound that causes a 50% decrease in binding:

<b>Cross-Reactant</b>	<b>Cross-reactivity</b>
Estriol-3-Sulphate	0.39%
Estriol-3-Glucoronide	0.14%
Estriol-16-Glucoronide	0.01%
Estrone-Sulphate	n.d. 10 µg/ml
Estrone-Glucoronide	n.d. 10 µg/ml
Estrone	0.10%
Estradiol	0.27%
16-Epiestriol	1.79%
17-Epiestriol	0.05%
16,17-Epiestriol	0.0%
16 $\alpha$ -Hydroxy Estrone	2.39%
Cheto-Estradiol	0.29%
Progesterone	n.d. 10 µg/ml
Hydroxyprogesterone	n.d. 10 µg/ml
DHEA	n.d. 10 µg/ml
Dexamethasone	n.d. 10 µg/ml
Cholesterol	n.d. 10 µg/ml
Cortisol	n.d. 10 µg/ml