17β-Estradiol RIA (CT)

Radioimmunoassay (Coated Tube)
for the quantitative determination of 17β-Estradiol
in human serum and plasma.

REF MG12101

Σ 96

2-8°C

EU: IVD CE

For research use only.
Not for use in diagnostic procedures.
Read entire protocol before use.

E2-RIA-CT

I. INTENDED USE
Radioimmunoassay for the \textit{in vitro} quantitative measurement of human Estradiol (E2) in serum and plasma.

II. GENERAL INFORMATION

A. Proprietary name : E2-RIA-CT Kit
B. Catalog number : MG12101 : 96 tests

III. CLINICAL BACKGROUND

A. Biological activity
17-beta-estradiol (E2) is a C-18 steroid hormone (molecular weight 272.4 Da) produced mainly by the ovary and placenta, and in small amounts by adrenals and testes. Estradiol is in equilibrium with estrone, which can be converted to estriol by the liver and placenta.

B. Clinical applications
Like for LH-FSH-progesterone, measurement of estradiol concentration in serum, peritoneal fluid and follicular fluid is an essential biochemical tool for the investigation of fertility, tumor and sexual diseases, and disorders of hypothalamic/pituitary/gonadal axis, for example :
- To detect the follicular phase;
- To check the effectiveness of the induction of ovulation (with ultrasound) and the level of E2 in follicular fluid makes it possible to detect normal or dysfunctional ovulation induction (the empty follicle syndrome may reflect a dysfunctional ovulation induction);
- To diagnose the luteinized unruptured follicle (LUF) syndrome (by the estimation of 17 beta-estradiol and progesterone levels in peritoneal fluid);
- To aid in the diagnosis of breast tumors (total estrogens - E1-E2 - and 17 beta-hydroxysteroid dehydrogenase activity are significantly higher in malignant than in non malignant breast tissues);
- With LH-FSH and E2 levels, it is possible to suspect a Stein Cohen-Leventhal syndrome;
- Other areas of investigation are : premature adrenarche, gynecomastie and menopausal period.
IV. PRINCIPLES OF THE METHOD

A fixed amount of ¹²⁵I labelled steroid competes with the steroid to be measured present in the sample or in the calibrator for a fixed amount of antibody sites immobilized on the wall of a polystyrene tube. Neither extraction nor chromatography is required because of the high specificity of the coated antibodies. After 3 hours incubation at 37°C, an aspiration step terminates the competition reaction. The tubes are then washed with 3 ml of washing solution and aspirated. A calibration curve is plotted and the E2 concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

<table>
<thead>
<tr>
<th>Reagents</th>
<th>96 Test Kit</th>
<th>Colour Code</th>
<th>Reconstitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubes coated with anti E2</td>
<td>2 x 48</td>
<td>brown</td>
<td>Ready for use</td>
</tr>
<tr>
<td>Ag ¹²⁵I CONC</td>
<td>1 vial</td>
<td>red</td>
<td>Transfer quantitatively the ethanol solution in the tracer buffer</td>
</tr>
<tr>
<td>TRACER: ¹²⁵Iodine labelled E2 (HPLC grade) in ethanol solution</td>
<td>1 vial 105 ml</td>
<td>black</td>
<td>Ready for use</td>
</tr>
<tr>
<td>TRACER BUFFER</td>
<td>1 vial 105 ml</td>
<td>black</td>
<td>Ready for use</td>
</tr>
<tr>
<td>Tracer Buffer with bovine gelatin and azide (&lt;0.1%)</td>
<td>1 vial 105 ml</td>
<td>black</td>
<td>Ready for use</td>
</tr>
<tr>
<td>CAL 0</td>
<td>1 vial 5 ml</td>
<td>yellow</td>
<td>Ready for use</td>
</tr>
<tr>
<td>Zero calibrator in human serum and azide (0.5%)</td>
<td>6 vials 1 ml</td>
<td>yellow</td>
<td>Ready for use</td>
</tr>
<tr>
<td>CAL N</td>
<td>1 vial 10 ml</td>
<td>brown</td>
<td>Dilute 70 x with distilled water (use a magnetic stirrer).</td>
</tr>
<tr>
<td>Calibrators E2 N = 1 to 6 (see exact values on vial labels) in human serum and azide (0.5%)</td>
<td>2 vials lyophilized</td>
<td>silver</td>
<td>Add 1 ml distilled water</td>
</tr>
<tr>
<td>WASH SOLN CONC</td>
<td>1 vial 10 ml</td>
<td>brown</td>
<td>Dilute 70 x with distilled water (use a magnetic stirrer).</td>
</tr>
<tr>
<td>Wash solution (TRIS-HCl)</td>
<td>2 vials lyophilized</td>
<td>silver</td>
<td>Add 1 ml distilled water</td>
</tr>
</tbody>
</table>

Note: Use the zero calibrator for sample dilutions.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:
1. Distilled water
2. Pipettes for delivery of: 100µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Water bath at 37°C
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Any gamma counter capable of measuring ¹²⁵I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

A. Tracer: Transfer quantitatively the ethanol solution into the tracer buffer and mix.

B. Controls: Reconstitute the controls with 1 ml distilled water.

C. Working Wash solution: Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, controls are stable for one week at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 3 months. Avoid successive freezing and thawing.
- Freshly prepared Working Wash solution should be used on the same day.
- The tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alternations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma samples must be kept at 2-8°C.
- If the test is not run within 24 hrs, storage in aliquots at -20°C is recommended.
- Avoid successive freezing and thawing.
- Serum and heparinized plasma provide similar results:
  \[
  Y \text{(Serum)} = 0.95 \times (\text{hep. plasma}) + 3 \quad r = 0.98 \quad n = 16
  \]
- EDTA plasma provides 25% lower results than serum and heparinized plasma:
  \[
  Y \text{(Serum)} = 1.27 \times (\text{EDTA plasma}) + 12 \quad r = 0.98 \quad n = 16
  \]

X. PROCEDURE

A. Handling notes
   Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots. Bring all the reagents to room temperature prior to use.
   Thoroughly mix all reagents and samples by gentle agitation or swirling.
   In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
   High precision pipettors or automated pipetting equipment will improve the precision. Respect the incubation times.
   Prepare a calibration curve for each run, do not use data from previous runs.

B. Procedure
   1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes.
   2. Briefly vortex calibrators, controls and samples and dispense 100µl of each reagent and sample into respective tubes.
   3. Dispense 1000 µl of ¹²⁵Iodine labelled E2 into each tube, including the uncoated tubes for total counts.
   4. Shake the tube rack gently by hand to liberate any trapped air bubbles.
   5. Incubate for 3 hours at 37°C in a water bath.
   6. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
   7. Wash tubes with 3 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
   8. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
   9. Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula:

\[
\frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} = B/B0(\%)
\]

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B0(%) values for each calibrator point as a function of the E2 concentration of each calibrator point. Reject obvious outliers.
4. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
5. By interpolation of the sample (B/B0 ( %) values, determine the E2 concentrations of the samples from the calibration curve.
6. For each assay, the percentage of total tracer bound in the absence of unlabelled E2 (B0/T) must be checked.
The following data are for illustration only and should never be used instead of the real time calibration curve.

### XII. TYPICAL DATA

The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was 2 pg/ml.

### XIII. PERFORMANCE AND LIMITATIONS

#### A. Detection limit
Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was 2 pg/ml.

#### B. Specificity
The percentages of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone</td>
<td>1.8</td>
</tr>
<tr>
<td>Estradiol</td>
<td>1.2</td>
</tr>
<tr>
<td>Ethynylestradiol</td>
<td>0.8</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.0002</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.0012</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.0011</td>
</tr>
<tr>
<td>DHEA-sulphate</td>
<td>0.0001</td>
</tr>
<tr>
<td>Estradiol-3-glucuronide</td>
<td>1.4</td>
</tr>
<tr>
<td>Estradiol-17-glucuronide</td>
<td>0.1</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.0011</td>
</tr>
<tr>
<td>Equilin</td>
<td>0.2</td>
</tr>
<tr>
<td>Estradiol-17-Valerate</td>
<td>0.3</td>
</tr>
<tr>
<td>Estrone-3-glucuronide</td>
<td>&lt; 0.002</td>
</tr>
<tr>
<td>Estradiol-3-glucuronide</td>
<td>&lt; 0.003</td>
</tr>
<tr>
<td>Norgestrel</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>17a estradiol</td>
<td>0.5</td>
</tr>
<tr>
<td>Androstenediol-sulfate</td>
<td>0.4</td>
</tr>
<tr>
<td>Androstenediol</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

### C. Precision

#### INTRA-ASSAY PRECISION

| Serum | N | <X> ± SD (pg/ml) | CV (%) | | Serum | N | <X> ± SD (pg/ml) | CV (%) |
|-------|---|-----------------|--------||-------|---|-----------------|--------|
| A     | 20 | 250 ± 15        | 5.9    | | A     | 20 | 146 ± 15        | 10.1   |
| B     | 20 | 1131 ± 55       | 4.9    | | B     | 20 | 320 ± 20        | 6.2    |

SD: Standard Deviation; CV: Coefficient of variation

#### INTER-ASSAY PRECISION

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Theoretical Concent. (pg/ml)</th>
<th>Measured Concent. (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/1</td>
<td>3040.0</td>
<td>3040.0</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>1520.0</td>
<td>1520.0</td>
</tr>
<tr>
<td></td>
<td>1/4</td>
<td>760.0</td>
<td>760.0</td>
</tr>
<tr>
<td></td>
<td>1/8</td>
<td>380.0</td>
<td>380.0</td>
</tr>
<tr>
<td></td>
<td>1/16</td>
<td>190.0</td>
<td>190.0</td>
</tr>
<tr>
<td></td>
<td>1/32</td>
<td>95.0</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>1/64</td>
<td>47.5</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td>1/128</td>
<td>23.8</td>
<td>23.8</td>
</tr>
</tbody>
</table>

Samples were diluted with the zero calibrator.
XVI. PRECAUTIONS AND WARNINGS

Safety
For in vitro diagnostic use only.
This kit contains $^{125}$I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations. This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

XVII. BIBLIOGRAPHY

1. ALPER M et al (1987)
Comparison of follicular fluid hormones in patients with one or two ovaries participating in a program of in vitro fertilization.
Fertil. and Steril., 48, 1, 94-97.

2. GERRIS J. et al. (1986)
A lesion from IVF endocrinology: the importance of the follicular phase to success and failure in non IVF cycle.
Acta. Eur. Fertil. 17, 4, 251-258

Diagnosis of luteinized unruptured follicle by ultrasound and steroid hormone assays in peritoneal fluid: a comparative study.
Fertil. and Steril. 46, 5, 823-827

Subcellular concentrations of estrone, estradiol, androstenedione and 17-β-hydroxysteroid dehydrogenase (17BOH-SOH) Activity in malignant and non malignant human breast tissues.
Int. J. Cancer 40, 305-308

Outcome of in vitro fertilization in women with low response to ovarian stimulation.
Fertil. and Steril. 47, 5, 812-815.

Dose dependent response of symptoms, pituitary, and bone to transferma oestrogen in postmenopausal women.
Br.Med. 293, 1337-1339.

Pregnancy rate in relation to number of cleaved eggs replaced after in vitro fertilization in stimulated cycles monitored by serum levels of estradiol and progesterone as sole index.
Hum. Reprod. 4, 325-328.

XVII. SUMMARY OF THE PROTOCOL

<table>
<thead>
<tr>
<th>TOTAL COUNTS µl</th>
<th>CALIBRATORS µl</th>
<th>SAMPLE(S) CONTROLS µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrators (0-6)</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Samples, controls</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tracer</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Incubation
3 hours at 37°C in a water bath

Separation
Working Wash solution aspirate carefully

<table>
<thead>
<tr>
<th>Separation</th>
<th>Count tubes for 60 seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalogue Nr :</td>
<td>MG12101</td>
</tr>
<tr>
<td>P.I. Number :</td>
<td>1700461/en</td>
</tr>
<tr>
<td>Revision nr :</td>
<td>060901/1</td>
</tr>
</tbody>
</table>

Revision date : 2006-09-01
LIABILITY: Complaints will only be accepted in written and if all details of the test performance and results are included (complaint form available from IBL or supplier). Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2008-10-01