

Read entire protocol before use.

E2-RIA-CT

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles – Belgium

Not be used for risk calculation of Trisomie 21.

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of human Estradiol (E2) in serum and plasma.

II. GENERAL INFORMATION

A. Name: Bio-Line **Estradiol-Ria** Kit
B. Catalogue number : **BL-21-CT**: 100 tests
C. Manufactured by : Bio-Line S.A.
Rue André Fauchille.17 - B-1150 Bruxelles - Belgium
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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

Reagents	100Test Kit	Colour Code	Reconstitution			
 Tubes coated with anti E2	2 x 50	brown	Ready for use			
<table border="1" data-bbox="76 600 284 645"> <tr> <td>Ag</td> <td>¹²⁵I</td> <td>CONC</td> </tr> </table> TRACER: ¹²⁵ Iodine labelled E2 (HPLC grade) in ethanol solution	Ag	¹²⁵ I	CONC	1 vial 1 ml 142 kBq	red	Transfer quantitatively the ethanol solution in the tracer buffer
Ag	¹²⁵ I	CONC				
<table border="1" data-bbox="127 712 319 745"> <tr> <td>TRACER</td> <td>BUF</td> </tr> </table> Tracer Buffer with bovine gelatin and azide (<0.1%)	TRACER	BUF	1 vial 105 ml	black	Ready for use	
TRACER	BUF					
<table border="1" data-bbox="92 813 226 846"> <tr> <td>CAL</td> <td>0</td> </tr> </table> Zero calibrator in human serum and azide (0.5%)	CAL	0	1 vial 5 ml	yellow	Ready for use	
CAL	0					
<table border="1" data-bbox="92 913 226 947"> <tr> <td>CAL</td> <td>N</td> </tr> </table> Calibrators E2 N = 1 to 6 (see exact values on vial labels) in human serum and azide (0.5%)	CAL	N	6 vials 1 ml	yellow	Ready for use	
CAL	N					
<table border="1" data-bbox="188 1037 284 1137"> <tr> <td>WASH</td> </tr> <tr> <td>SOLN</td> </tr> <tr> <td>CONC</td> </tr> </table> Wash solution (TRIS-HCl)	WASH	SOLN	CONC	1 vial 10 ml	brown	Dilute 70 x with distilled water (use a magnetic stirrer).
WASH						
SOLN						
CONC						
<table border="1" data-bbox="76 1193 274 1238"> <tr> <td>CONTROL</td> <td>N</td> </tr> </table> Controls - N = 1 or 2 in human serum and thymol	CONTROL	N	2 vials lyophilized	silver	Add 1 ml distilled water	
CONTROL	N					

Note : Use the zero calibrator for sample dilutions.

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

- Distilled water
- Pipettes for delivery of: 100µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- Water bath at 37°C
- 5 ml automatic syringe (Cornwall type) for washing
- Aspiration system (optional)
- Any gamma counter capable of measuring ¹²⁵I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

- Tracer** : Transfer quantitatively the ethanol solution into the tracer buffer and mix.
- Controls**: Reconstitute the controls with 1 ml distilled water.
- 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.
- Alterations 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 pipettes 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

- Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes.
- Briefly vortex calibrators, controls and samples and dispense 100µl of each into respective tubes.
- Dispense 1000 µl of ¹²⁵Iodine labelled E2 into each tube, including the uncoated tubes for total counts.
- Shake the tube rack gently by hand to liberate any trapped air bubbles.
- Incubate for 3 hours at 37°C.
- 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.
- 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.
- Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
- Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

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

$$B/B0 (\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

- 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.
- 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.
- By interpolation of the sample (B/B0 (%)) values, determine the E2 concentrations of the samples from the calibration curve.
- For each assay, the percentage of total tracer bound in the absence of unlabelled E2 (B0/T) must be checked.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

E2-RIA-CT		cpm	B/Bo (%)
Total count		42224	
Calibrator	0 pg/ml	14082	100.0
	14 pg/ml	13338	94.7
	53 pg/ml	9800	69.6
	160 pg/ml	6231	44.2
	574 pg/ml	3008	21.4
	2300 pg/ml	1314	9.3
	3523 pg/ml	933	5.9

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 10 pg/ml.

B. Specificity

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

Compound	Cross-Reactivity (%)
Estrone	1.8
Estriol	1.2
Ethinylestradiol	0.8
Progesterone	0.0002
Testosterone	0.0012
Androstenedione	0.0011
DHEA-sulphate	0.0001
Estradiol-3-glucuronide	1.4
Estradiol-17-glucuronide	0.1
Cortisol	0.0011
Equilin	0.2
Estradiol-17-Valerate	0.3

D. Precision

E.

INTRA-ASSAY PRECISION

INTER-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

D. Accuracy

DILUTION TEST

Sample	Dilution	Theoretical Concent. (pg/ml)	Measured Concent. (pg/ml)
1	1/8	2719	2701
	1/10	2175	2157
	1/20	1088	1009
	1/50	435	433
	1/100	218	235
	1/200	109	110
	1/500	44	44
	1/1000	22	22
2	1/1	-	490
	1/2	245	258
	1/4	123	148
	1/5	103	110
	1/8	61	65
	1/10	52	59
	1/16	31	31
	1/32	15	16

Samples were diluted with the zero calibrator.

RECOVERY TEST

Sample	added E2 (pg/ml)	Recovered E2 (pg/ml)	Recovered (%)
1	19.5	23	118
	32	34	106
	74.5	54	73
	222	203	91
	662	646	98
	1412	1353	96
2	673.5	607	90
	686	555	81
	728.5	666	91
	876	846	97
	1316	1367	104
	2066	2199	106

Conversion factor :

From ng/ml to nmol/L : x 3.68

From nmol/L to ng/ml : x 0.272

The concentrations of the calibrators are determined with the ID-GC/MS reference method.

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrator has been added to coated tubes.

TIME DELAY

Serum (pg/ml)	0'	10'	20'	30'
S1	158	170	156	152
S2	319	314	320	314

XIV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

XV. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

	Concentration range (2.5 to 97.5% percentiles) (pg/ml)	Number of subjects
Normal males	9 - 53	18
Normal Females		
· Follicular phase (day -10 to -3)	36 - 251	31
· Preovulatory phase (day -1 & 0)	93 - 508	54
· Luteal phase (day 3 to 10)	52 - 257	37
· Postmenopausal	3 - 30	50
Pregnancy		
· First trimester	510 - 6300	20
· Second trimester	2400 - 18900	26
· Third trimester	11900 - 37100	20

XVI. PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only.

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

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XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS μ l	CALIBRATORS μ l	SAMPLE(S) CONTROLS μ l
Calibrators (0-6)	-	100	-
Samples, controls	-	-	100
Tracer	1000	1000	1000
Incubation	3 hours at 37°C		
Separation	-	aspirate	
Working Wash solution		3.0 ml	
Separation		aspirate carefully	
Counting	Count tubes for 60 seconds		

Bio-Line Catalogue Nr : BL-21-CT	Version: 040702-BL	Revision nr : 040127/1
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