



E2-RIA-CT

KIP0629

LOT : 151006/4

Read entire protocol before use.

E2-RIA-CT

I. INTENDED USE

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

II. GENERAL INFORMATION

- A. **Proprietary name :** DIAsource E2-RIA-CT Kit
- B. **Catalog number :** KIP0629 : 96 tests
- C. **Manufactured by :** DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

For technical assistance or ordering information contact :
Tel : +32 (0)10 84.99.11 Fax : +32 (0)10 84.99.91

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 adrenarache, gynecomastie and menopausal period.

IV. PRINCIPLES OF THE METHOD

A fixed amount of ^{125}I 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	96 Test Kit	Colour Code	Reconstitution			
 Tubes coated with anti E2	2 x 48	brown	Ready for use			
<table border="1" data-bbox="76 571 284 622"> <tr> <td>Ag</td> <td>^{125}I</td> <td>CONC</td> </tr> </table> TRACER: ^{125}I 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="103 712 295 757"> <tr> <td>TRACER</td> <td>BUF</td> </tr> </table> Tracer Buffer with bovine gelatin and azide (<0.1%)	TRACER	BUF	1 vial 53 ml	black	Ready for use	
TRACER	BUF					
<table border="1" data-bbox="108 817 247 862"> <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="108 922 247 967"> <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="71 1064 327 1108"> <tr> <td>WASH</td> <td>SOLN</td> <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="114 1160 311 1205"> <tr> <td>DIL</td> <td>SPE</td> </tr> </table> Samples diluent: human serum and azide (0.5%)	DIL	SPE	1 vial 2.5 ml	black	Ready for use	
DIL	SPE					
<table border="1" data-bbox="95 1281 295 1326"> <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 0.5 ml distilled water	
CONTROL	N					

VI. SUPPLIES NOT PROVIDED

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

- Distilled water
- Pipettes for delivery of: $50\mu\text{L}$ and $500\mu\text{L}$ (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 ^{125}I 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 0.5 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 samples must be kept at $2-8^\circ\text{C}$.
- If the test is not run within 24 hrs, storage in aliquots at -20°C is recommended.
- Avoid successive freezing and thawing.
- Dilution of high concentration samples: the provided diluent contains a small amount of Estradiol, and has to be tested to determinate this concentration. This value has to be subtracted from the concentration of the samples before multiplying the results by the dilution factor.

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 $50\mu\text{L}$ of each into respective tubes.
- Dispense $500\mu\text{L}$ of ^{125}I Iodine 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 in a water bath.
- 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 :

$$B/B_0(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

- 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	69089	
Calibrator		
0 pg/ml	29372	100.0
9 pg/ml	25237	85.9
27 pg/ml	21374	72.7
92 pg/ml	15669	53.4
430 pg/ml	8527	29.0
2200 pg/ml	2607	8.9
3900 pg/ml	1676	5.7

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit

Twenty two 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 2.7 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	1.0
Estriol	0.6
Ethinylestradiol	0.2
Progesterone	<0.0002
Testosterone	<0.001
Androstenedione	<0.001
DHEA-sulphate	<0.0002
Estradiol-17-glucuronide	<0.2
Cortisol	<0.001
Equilin	<0.1
Estradiol-17-Valerate	<0.1
Norgestrel	<0.0004
Androstendiol	0.001

C. Precision

INTRA-ASSAY PRECISION

INTER-ASSAY PRECISION

Serum	N	<X> ± SD (pg/ml)	CV (%)	Serum	N	<X> ± SD (pg/ml)	CV (%)
A	20	80 ± 6.4	8.0	A	23	79 ± 11	13.9
B	20	141 ± 7.9	5.6	B	23	249 ± 26	10.4
C	20	249 ± 11.7	4.7				

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

DILUTION TEST

Dilution	Theoretical Concent. (pg/ml)	Measured Concent. (pg/ml)	Recovered (%)
1/1	1400.3	1387.3	100
1/2	700.2	659.0	96
1/4	350.1	358.0	106
1/8	175.1	158.0	98
1/16	87.5	64.9	89
1/32	43.8	29.3	97
1/64	21.9	11.9	114

The sample was diluted with the provided diluent. The endogenous Estradiol in the diluent was 13 pg/ml and this value was deducted from the sample concentration before applying the dilution factor.

Measured and theoretical concentrations were consistent:

$$Y (\text{measured}) = 0.99X (\text{theoretical}) - 0 ; R^2 = 0.999$$

RECOVERY TEST

Sample	Added E2 (pg/ml)	Theoretical E2 (pg/ml)	Measured E2 (pg/ml)	Recovered (%)
1	0	12.8	12.8	NA
	4.5	17.3	18.3	106
	13.5	26.3	22.3	85
	46.0	58.8	54.4	93
	215	227.8	207	91
	1100	1112.8	1104	99
	1950	1962.8	2259	115

Measured and theoretical concentrations were consistent:

$$- \text{Sample 1: } Y (\text{measured}) = 1.11X (\text{theoretical}) + 0 ; R^2 = 0.999$$

Conversion factor :

$$\text{From ng/ml to nmol/L : } x 3.68$$

$$\text{From nmol/L to ng/ml : } x 0.272$$

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 40 minutes after the calibrator has been added to coated tubes.

TIME DELAY

Serum (pg/ml)	0'	10'	20'	30'	40'
C1	63	58	79	69	75
C2	217	229	274	235	237

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 of 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)	Median concentration (pg/ml)	Number of subjects
Normal males	8 - 66	27	36
Normal Females			
· Follicular phase (day -10 to -3)	35-147	69	49
· Preovulatory phase (day -1 & 0)	Still under determination		
· Luteal phase (day 3 to 10)	43 - 217	105	35
· Postmenopausal	19 - 49	31	39

XVI. PRECAUTIONS AND WARNINGS

Safety

For in vitro diagnostic use only.

This kit contains ¹²⁵I (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

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Hum. Reprod. 4, 325-328.

XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS μ l	CALIBRATORS μ l	SAMPLE(S) CONTROLS μ l
Calibrators (0-6)	-	50	-
Samples, controls	-	-	50
Tracer	500	500	500
Incubation	3 hours at 37°C in a water bath		
Separation	-	aspirate	
Working Wash solution		3.0 ml	
Separation		aspirate carefully	
Counting	Count tubes for 60 seconds		

DIAsource Catalogue Nr : KIP0629	P.I. Number : 1700461/en	Revision nr : 151006/4
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Revision date : 2015-10-30

Distributed by:



Immuno-Biological Laboratories, Inc. (IBL-America)

8201 Central Ave. NE, Suite P
Minneapolis, MN 55432, USA
Phone: (888) 523-1246
Fax.: (763) 780-2988
Web: www.ibl-america.com
Email: info@ibl-america.com

	Used symbols
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
I V D	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 125I	Tracer
Ab 125I	Tracer
Ag 125I CONC	Tracer concentrated
Ab 125I CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoabsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
µT	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
PREC AGENT	Precipitating Agent
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor
TRAY	Incubation trays
PMSF	PMSF solution
	Protect from light
STRIP	Dot Strip
SUB	Substrate
EXTR SOLN CONC	Extraction Buffer Concentrate
CART	Cartridge
SAV HRP	Streptavidin HRP
PIPETTE	Pipette
WASH SOLN	Wash buffer