Estradiol II

cobas®

REF	Σ	SYSTEM
03000079 190	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

For USA: Elecsys Estradiol II Assay

English

System information

For **cobas e** 411 analyzer: test number 101
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 012

Intended use

Immunoassay for the in vitro quantitative determination of estradiol in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Estrogens are responsible for the development of the secondary female sex characteristics. Together with gestagens they control all the important female reproductive processes.

The biologically most active estrogen is 17β-estradiol. This is a steroid hormone having a molecular weight of 272 daltons.

Estrogens are produced primarily in the ovary (follicle, corpus luteum), but small quantities are also formed in the testes and in the adrenal cortex. During pregnancy, estrogens are mainly formed in the placenta. About 98 % of estradiol is bound to transport proteins (SHBG = sex hormone binding globulin).[1]

Estrogen secretion is biphasic during the menstrual cycle. The determination of estradiol is utilized clinically in the elucidation of fertility disorders in the hypothalamus-pituitary-gonad axis, gynecomastia, estrogen-producing ovarian and testicular tumors and in hyperplasia of the adrenal cortex. Further clinical indications are the monitoring of fertility therapy and determining the time of ovulation within the framework of in vitro fertilization (IVF).[2,3,4]

The Elecsys Estradiol II assay employs a competitive test principle using a polyclonal antibody specifically directed against 17β-estradiol. Endogenous estradiol released from the sample by mesterolone competes with the added estradiol derivative labeled with a ruthenium complex[a)] for the binding sites on the biotinylated antibody.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating the sample (35 µL) with an estradiol-specific biotinylated antibody, an immunocomplex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 2nd incubation: After addition of streptavidin-coated microparticles and an estradiol derivative labeled with a ruthenium complex, the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reagents - working solutions

The reagent rackpack is labeled as E2 II.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-estradiol-Ab~biotin (gray cap), 1 bottle, 8 mL:

Biotinylated polyclonal anti-estradiol antibody (rabbit) 45 ng/mL; Mesterolone 130 ng/mL; MES buffer 50 mmol/L, pH 6.0; preservative.

R2 Estradiol-peptide~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL:

Estradiol derivative, labeled with ruthenium complex 2.75 ng/mL; MES buffer 50 mmol/L, pH 6.0; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

For USA: For prescription use only.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	56 days (8 weeks)
on the analyzers	56 days (8 weeks)

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma as well as plasma separation tubes.

Criterion: Recovery within 70-130 % of serum value > 100 pg/mL, recovery of ± 20 pg/mL of serum value ≤ 100 pg/mL and slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 2 days at 2-8 °C, 6 months at -20 °C. Freeze only once.[5]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03064921122, Estradiol II CalSet II, for 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 4 x 3 mL
- REF 11731416160, PreciControl Universal, for 4 x 3 mL (for USA)
- REF 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution
- REF 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized via ID-GC/MS ("isotope dilution-gas chromatography/mass spectrometry").[6]

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L, pg/mL, ng/L or additionally in nmol/L with MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Conversion factors:	pmol/L x 0.273 = pg/mL (ng/L)
	pg/mL x 3.67 = pmol/L

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1000 mg/dL) and biotin (< 147 nmol/L or < 36 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1200 IU/mL.

In vitro tests were performed on 18 commonly used pharmaceuticals. No interference with the assay was found.

Erroneous test results may be obtained from samples taken from patients who have been exposed to vaccines containing rabbit serum or when keeping rabbits as pet animals.

Due to the risk of cross reactivity, this assay should not be used when monitoring Estradiol levels in patients being treated with Fulvestrant.

Steroid drugs may interfere with this test.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

18.4-15781 pmol/L (5.00-4300 pg/mL) (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 18.4 pmol/L or < 5.00 pg/mL. Values above the measuring range are reported as > 15781 pmol/L or > 4300 pg/mL (or up to 78905 pmol/L or 21500 pg/mL for 5-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 18.4 pmol/L (5.00 pg/mL)

The lower detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

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Dilution

Samples with estradiol concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:5 (automatically by the analyzers). The concentration of the diluted sample must be > 1835 pmol/L (> 500 pg/mL).

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

The endogenous analyte concentration of the diluent (< 220 pmol/L or < 60.0 pg/mL) is not taken into account for dilutions above the measuring range.

Expected values

Studies with the Elecsys Estradiol II assay conducted in four clinical centers in Germany and Austria covering a total of 520 samples from healthy individuals gave the following reference values listed below (study No.: B00P023 and C00P032 - status December 2001):

Test subjects	N	Percentiles			
		50th	5-95th	50th	5-95th
		pmol/L		pg/mL	
Men	109	76.2	28.0-156	20.8	7.63-42.6
Women					
• Follicular phase	88	228	46.0-607	62.2	12.5-166
• Ovulation phase	49	812	315-1828	221	85.8-498
• Luteal phase	83	389	161-774	106	43.8-211
• Postmenopause	32	44.0	< 18.4-201*	12.0	< 5.00-54.7*
Pregnancy					
• 1st trimester	20	3685	789-> 15781	1004	215-> 4300
Children (1-10 years)					
• Boys	74	40.4	< 18.4-73.4*	11.0	< 5.00-20.0*
• Girls	65	47.7	22.0-99.1	13.0	6.00-27.0

*18.4 pmol/L (5.00 pg/mL) is the lower detection limit of the test.

Details are available on request and also contained in the Elecsys Estradiol II product information.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	pmol/L	pg/mL	pmol/L	pg/mL	%	pmol/L	pg/mL	%
HS[b] 1	149	40.6	6.37	1.74	4.3	14.8	4.02	9.9
HS 2	334	91.1	8.03	2.19	2.4	19.4	5.29	5.8
HS 3	3337	909	125	34.0	3.7	143	39.0	4.3
HS 4	10639	2899	341	93.0	3.2	651	177	6.1
HS 5	13785	3756	631	172	4.6	874	238	6.3
PC U[c]1	345	94.1	14.1	3.84	4.1	24.4	6.66	7.1
PC U2	2026	552	83.5	22.8	4.1	99.6	27.1	4.9

b) HS = human serum

c) PC U = PreciControl Universal

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	pmol/L	pg/mL	pmol/L	pg/mL	%	pmol/L	pg/mL	%
HS 1	143	39.1	8.71	2.37	6.1	10.0	2.73	7.0
HS 2	292	79.6	10.1	2.75	3.5	13.1	3.58	4.5
HS 3	3220	878	41.7	11.4	1.3	61.5	16.8	1.9
HS 4	10309	2809	221	60.2	2.1	294	80.0	2.8
HS 5	13961	3804	333	90.7	2.4	522	142	3.7
PC U1	378	103	9.87	2.69	2.6	14.2	3.88	3.8
PC U2	2037	555	38.2	10.4	1.9	42.6	11.6	2.1

Method comparison

Method comparison of the Elecsys Estradiol II assay (y) in 34 samples measured with ID-GC/MS (x) - pg/mL:

Passing/Bablok[7]	Linear regression
y = 0.986x + 4.15	y = 0.921x + 28.1
τ = 0.989	r = 0.996

The sample concentrations were between approximately 14.1 and 11667 pmol/L (approximately 3.83 and 3179 pg/mL).

Analytical specificity

For the Estradiol II assay, the following cross-reactivities were found (in %):

a) Substance added per 0.1 µg/mL:

Aldosterone	0.005
Androstendione	0.007
Equiline	0.071
Estriol	0.218
Estrone	0.811
Estrone-3β-glucuronide	0.002
Estrone-3-sulfate	0.006
Ethisterone	0.005
Norethindrone acetate	0.014
Pregnenolone	0.003
Progesterone	0.001
2-Methoxy-estradiol	0.077
17β-Estradiol-3,17-sulfate	0.867
17β-Estradiol-3-β-D-glucuronide	0.126
17β-Estradiol-17-β-D-glucuronide	0.055
17β-Estradiol-3-glucuronide-17-sulfate	0.011
17β-Estradiol-3-sulfate-17-glucuronide	0.001
17β-Estradiol-3-sulfate	0.286
17β-Estradiol-17-valerate	0.142
17β-Estradiol-17-sulfate	0.002
17-Hydroxyprogesterone	0.001

b) Substance added per 0.2 µg/mL:

Cortisol	0.001
Cortisone	n.d.[d]
Tamoxifen	0.004

17-α-Ethinyl-estradiol	0.231

d) n.d. = not detectable

c) Substance added per 0.25 µg/mL:

Chlomiphene	0.001

d) Substance added per 1.0 µg/mL:

Prednisolone	n.d.

e) Substance added per 10 µg/mL:

Danazol	0.001
DHEA-S	n.d.
Mesterolone	n.d.
Testosterone	0.001
5-α-Dihydrotestosterone	0.001
5-Androstene-3β-,17β-diol	0.001

Functional sensitivity

44 pmol/L (12 pg/mL)

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

References

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6 Thienpont LM, Verhseghe PG, Van Brussel KA, et al. Estradiol-17-beta quantified in serum by isotope dilution-gas chromatography-mass spectrometry. Clin Chem 1988(34);10:2066-2069.

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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