

REF		SYSTEM
11775863 122	100	Elecsys 2010 MODULAR ANALYTICS E170 cobas e 411 cobas e 601 cobas e 602

English**Intended use**

Immunoassay for the in vitro quantitative determination of follicle-stimulating hormone in human serum and plasma.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

FSH (follicle stimulating hormone), together with LH (luteinizing hormone), belongs to the gonadotropin family. FSH and LH regulate and stimulate the growth and function of the gonads (ovaries and testes) synergistically.¹

Like LH, TSH and hCG, FSH is a glycoprotein consisting of two subunits (α - and β -chains). Its molecular weight is approx. 32000 daltons.

In women, the gonadotropins act within the hypothalamus-pituitary-ovary regulating circuit to control the menstrual cycle.^{2,3}

FSH and LH are released in pulses from the gonadotropic cells of the anterior pituitary. The levels of the circulating hormones are controlled by steroid hormones via negative feedback to the hypothalamus. In the ovaries FSH, together with LH, stimulates the growth and maturation of the follicle and hence also the biosynthesis of estrogens in the follicles.

The FSH level shows a peak at mid-cycle, although this is less marked than with LH. Due to changes in ovarian function and reduced estrogen secretion, high FSH concentrations occur during menopause.³

In men, FSH serves to induce spermatogonium development.

Determination of the FSH concentration is used in the elucidation of dysfunctions within the hypothalamus-pituitary-gonads system.

The determination of FSH in conjunction with LH is utilized for the following indications: congenital diseases with chromosome aberrations, polycystic ovaries (PCO), amenorrhea (causes), and menopausal syndrome. Depressed gonadotropin levels in men occur in azoospermia.^{1,3,4,5}

The Elecsys FSH assay employs two different monoclonal antibodies specifically directed against human FSH. Cross-reactivity with LH, TSH, hCG, hGH, and hPL is negligible.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 40 μ L of sample, a biotinylated monoclonal FSH-specific antibody, and a monoclonal FSH-specific antibody labeled with a ruthenium complex^{a)} form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the 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.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Reagents - working solutions

The reagent rackpack is labeled as FSH.

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-FSH-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-FSH antibody (mouse) 0.5 mg/L, MES buffer 50 mmol/L, pH 6.0; preservative.
- R2 Anti-FSH-Ab~Ru(bpy)₃²⁺ (black cap), 1 bottle, 10 mL: Monoclonal anti-FSH antibody (mouse) labeled with ruthenium complex 0.8 mg/L, 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.

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	12 weeks
on the analyzers	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested.

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

Li-, Na-, NH₄⁺-heparin, and K₃-EDTA plasma. When sodium citrate is used, the values obtained are by 20 % lower as compared to serum, those for sodium fluoride/potassium oxalate are by approx. 14 % lower.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within $\pm 2 \times$ analytical sensitivity (LDL) + coefficient of correlation > 0.95 .

Stable for 14 days at 2-8 °C, 6 months at -20 °C.⁶ Freeze only once.⁷

Stability of serum obtained with separating tubes: 48 hours at 2-8 °C (note the data provided by the tube manufacturer).

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.



Follicle-stimulating hormone

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] 03032680122, FSH CalSet II, for 4 x 1 mL
- [REF] 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- [REF] 11731416160, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2 (for USA)
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- [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, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- [REF] 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

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 Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- [REF] 03023150001, WasteLiner, waste bags
- [REF] 03027651001, SysClean Adapter M

Accessories for all analyzers:

- [REF] 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution
- [REF] 11298500160, 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.

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

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.

Bring the cooled reagents to approx. 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 against the Enzygum-Test FSH method. This in turn has been standardized against the 2nd IRP WHO reference standard 78/549.

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 mIU/mL or in IU/L).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1094 µmol/L or < 64 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1900 mg/dL) and biotin (< 246 nmol/L or < 60 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 2250 IU/mL.

There is no high-dose hook effect at FSH concentrations up to 2000 mIU/mL.

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

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**

0.100-200 mIU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as



Follicle-stimulating hormone

< 0.100 mIU/mL. Values above the measuring range are reported as > 200 mIU/mL.

Lower limits of measurement*Lower detection limit of the test*

Lower detection limit: < 0.100 mIU/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).

Dilution

Not necessary due to the broad measuring range.

Expected values

Studies with the Elecsys FSH assay have revealed the following FSH values:

Test subjects ^{b)}	N	FSH (mIU/mL)		
		Percentile		
		50 th	5 th	95 th
Men	319	4.6	1.5	12.4
Women				
• Follicular phase	376	6.9	3.5	12.5
• Ovulation phase	56	12.3	4.7	21.5
• Luteal phase	349	3.6	1.7	7.7
• Postmenopause	181	67.0	25.8	134.8

b) Reference ranges for children are available on request and are also contained in the Elecsys FSH product information.

LH/FSH quotient: Quotients have been calculated from the results obtained with the Elecsys LH assay and the Elecsys FSH assay in the samples of healthy women of child-bearing age. The following medians have been calculated:

Follicular phase: 0.82 (n = 315)

Luteal phase: 1.12 (n = 279)

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 modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 2010 and cobas e 411 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 1	1.2	0.02	1.8	0.06	5.3
Human serum 2	50.4	0.74	1.5	1.90	3.8
Human serum 3	103	1.85	1.8	5.24	5.1
PC ^{c)} Universal 1	11.1	0.22	2.0	0.41	3.7
PC Universal 2	28.9	0.40	1.4	0.85	2.9

c) PC = PreciControl

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers						
		Repeatability		Intermediate precision		
Sample	Mean mIU/mL	SD mIU/mL	CV %	Mean mIU/mL	SD mIU/mL	CV %
Human serum 1	5.97	0.15	2.6	5.33	0.19	3.6
Human serum 2	54.4	1.55	2.8	45.9	1.70	3.7
Human serum 3	178	4.54	2.5	229	10.3	4.5
PC Universal 1	9.53	0.14	1.5	8.29	0.33	4.0
PC Universal 2	24.6	0.31	1.3	21.6	0.84	3.9

Method comparison

A comparison of the Elecsys FSH assay (y) with the Enzygum-Test FSH method (x) using clinical samples gave the following correlations (mIU/mL):
Number of samples measured: 160

Passing/Bablok⁸ Linear regression
 $y = 1.093x + 0.213$ $y = 1.098x + 0.114$
 $r = 0.944$ $r = 0.998$

The sample concentrations were between approx. 0.65 and 152 mIU/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

LH, TSH, hCG, hGH and hPL < 0.1 %

References

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- 7 Wu AHB. Tietz Clinical Guide To Laboratory Tests, 4th Edition, WB Saunders Co, 2006: 412 pp.
- 8 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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



FSH

Follicle-stimulating hormone

SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

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