08946710500V1.0

Elecsys ACTH

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REF		Σ	SYSTEM
08946710190	08946710500	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 2340
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 97

Intended use

Immunoassay for the in vitro quantitative determination of adrenocorticotropic hormone (ACTH) in human EDTA plasma.

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

Summary

Adrenocorticotropic hormone or corticotropin is a peptide hormone consisting of 39 amino acids. It is produced in the anterior pituitary of the brain as part of the precursor molecule pro-opiomelanocortin (POMC). Tissue-specific cleavage results in ACTH and a range of related peptides.[1,2]

ACTH stimulates formation and secretion of glucocorticoids (especially cortisol) by the adrenal cortex.

The glucocorticoid production is regulated by various factors.[3,4,5,6] After stimulation (e.g. by physical effort or by the internal body clock), the hypothalamus secretes CRH (corticotropin releasing hormone). CRH acts on the pituitary, which in turn synthesizes and secretes ACTH. Finally, ACTH stimulates secretion of the glucocorticoids by the adrenals. High concentrations of glucocorticoids in the blood inhibit secretion of CRH and ACTH via a negative feedback mechanism.

ACTH concentrations show a diurnal variation with high levels in the morning and low levels in the evening. Therefore, as with cortisol, it is important to know the collection time of the plasma sample for interpretation of the results.

Plasma ACTH measurements are useful in the differential diagnosis of Cushing's disease (ACTH hypersecretion), autonomous ACTH producing pituitary tissue (e.g. Nelson's syndrome), hypopituitarism with ACTH deficiency and ectopic ACTH syndrome.[7,8] In addition to cortisol measurements, ACTH determinations can be used together with suppression or stimulation tests to diagnose the origin of glucocorticoid overproduction. Similarly, ACTH measurements can be employed to facilitate differential diagnosis of adrenocortical insufficiency (Addison's disease).[9]

ACTH not produced by the pituitary gland is known as ectopic ACTH;[10] this is often associated with small cell carcinoma of the lung. In rare cases ectopic ACTH can be caused by thymic tumors, pancreatic adenocarcinomas, or bronchial carcinoids. These tumors often secrete ACTH precursors (POMC and pro-ACTH).

The Elecsys ACTH assay employs 2 monoclonal antibodies specific for ACTH (9-12) and for the C-terminal region (ACTH 36-39).

Due to common antigenic structure, the antibodies recognize intact biologically active ACTH 1-39 and the ACTH precursors POMC and pro-ACTH.[2]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, a biotinylated monoclonal ACTH-specific antibody, and a monoclonal ACTH-specific antibody labeled with a ruthenium complex[a)] react to 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 or e-barcode.

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

Reagents - working solutions

The reagent rackpack is labeled as ACTH.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-ACTH-Ab~biotin (gray cap), 1 bottle, 8 mL: Biotinylated monoclonal anti-ACTH antibody (mouse) 0.3 mg/L; MES[b)] buffer 50 mmol/L, pH 6.2; preservative.

R2 Anti-ACTH-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL: Monoclonal anti-ACTH antibody (mouse) labeled with ruthenium complex 0.3 mg/L; MES buffer 50 mmol/L, pH 6.2; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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

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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	4 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

K_2- and K_3-EDTA plasma, collected using siliconized glass tubes or plastic tubes as ACTH adsorbs to non-siliconized glass tubes and thereby reduces sample ACTH values.[2] Do not use other types of plasma samples.

Criterion for K_2-EDTA plasma: slope 0.85-1.15 + coefficient of correlation ≥ 0.95 for method comparison vs K_3-EDTA plasma.

Only use pre-cooled sampling vials. After drawing the blood, put the vials immediately on ice. Use a cooled centrifuge to separate the plasma. Measure samples immediately or freeze them at -20 °C (± 5° C).

Stable for 3 hours at 2-8 °C followed by 2 hours at 20-25 °C, 10 weeks at -20 °C (± 5 °C). Freeze only once.

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 08959820190, ACTH CalSet, for 4 x 1.0 mL
- REF 05341787190, PreciControl Multimarker, for 6 x 2.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **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

Additional materials for **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

Additional materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

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.

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 gravimetrically with synthetic ACTH produced at Roche.

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).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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 Multimarker.

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.

If necessary, repeat the measurement of the samples concerned.

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

Calculation

The analyzer automatically calculates the analyte concentration of each sample either in pg/mL, pmol/L or ng/L (selectable).

Conversion factors: pg/mL x 0.2202 = pmol/L

pmol/L x 4.541 = pg/mL

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Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 428 µmol/L or ≤ 25 mg/dL
Hemoglobin	≤ 0.248 mmol/L or ≤ 400 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 400 IU/mL

Criterion: For concentrations of 1.5-20 pg/mL the deviation is ± 3 pg/mL. For concentrations > 20-2000 pg/mL the deviation is ± 15 %.

There is no high-dose hook effect at ACTH concentrations up to 1 x 10^6 pg/mL.

Pharmaceutical substances

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

However, under ACTH 1-24 medication, ACTH measurement is not recommended, due to negative interference with the sandwich assay.

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

1.5-2000 pg/mL or 0.330-440 pmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 1.5 pg/mL or < 0.330 pmol/L. Values above the measuring range are reported as > 2000 pg/mL or > 440 pmol/L.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 1.00 pg/mL (0.220 pmol/L)

Limit of Detection = 1.5 pg/mL (0.330 pmol/L)

Limit of Quantitation = 3.0 pg/mL (0.661 pmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

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

Dilution

Not necessary due to the broad measuring range.

Expected values

Studies with the Elecsys ACTH assay using plasma samples from 354 apparently healthy adults gave the following results (5th-95th percentile):

7.2-63.3 pg/mL (1.6-13.9 pmol/L)

The plasma samples were drawn between 7-10 a.m.

ACTH concentrations vary considerably depending on physiological conditions. Therefore, ACTH results should always be evaluated together with simultaneously measured cortisol concentrations.

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 plasma and controls in a protocol (EP05-A3) 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 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human plasma 1	4.11	0.119	2.9	0.156	3.8
Human plasma 2	24.6	0.294	1.2	0.441	1.8
Human plasma 3	45.6	0.494	1.1	0.638	1.4
Human plasma 4	894	7.09	0.8	13.8	1.5
Human plasma 5	1917	18.5	1.0	33.5	1.7
PC[c)] Multimarker 1	47.9	0.538	1.1	0.730	1.5
PC Multimarker 2	899	6.13	0.7	11.7	1.3

c) PC = PreciControl

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human plasma 1	0.905	0.026	2.9	0.034	3.8
Human plasma 2	5.42	0.065	1.2	0.097	1.8
Human plasma 3	10.0	0.109	1.1	0.141	1.4
Human plasma 4	197	1.56	0.8	3.04	1.5
Human plasma 5	422	4.07	1.0	7.38	1.7
PC Multimarker 1	10.5	0.119	1.1	0.161	1.5
PC Multimarker 2	198	1.35	0.7	2.58	1.3

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human plasma 1	4.08	0.102	2.5	0.127	3.1
Human plasma 2	25.6	0.322	1.3	0.375	1.5
Human plasma 3	47.2	0.517	1.1	0.634	1.3
Human plasma 4	891	8.90	1.0	13.8	1.6
Human plasma 5	1838	17.0	0.9	31.1	1.7
PC Multimarker 1	48.9	0.449	0.9	0.584	1.2
PC Multimarker 2	898	7.01	0.8	10.1	1.1

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cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human plasma 1	0.898	0.022	2.5	0.028	3.1
Human plasma 2	5.64	0.071	1.3	0.083	1.5
Human plasma 3	10.4	0.114	1.1	0.140	1.3
Human plasma 4	196	1.96	1.0	3.04	1.6
Human plasma 5	405	3.74	0.9	6.85	1.7
PC Multimarker 1	10.8	0.099	0.9	0.129	1.2
PC Multimarker 2	198	1.54	0.8	2.22	1.1

Method comparison

A comparison of the Elecsys ACTH assay, REF 08946710190 (**cobas e** 601 analyzer; y), with the Elecsys ACTH assay, REF 03255751190 (**cobas e** 601 analyzer; x), gave the following correlations (pg/mL):

Number of samples measured: 186

Passing/Bablok[11]	Linear regression
y = 0.988x + 0.234	y = 0.998x + 0.091
τ = 0.987	r = 0.999

The sample concentrations were between 2.07 and 1905 pg/mL.

Analytical specificity

The Elecsys ACTH 2-site immunoassay measures intact ACTH 1-39. When ACTH fragments or peptides were added to a patient's plasma sample with defined ACTH concentration, no interference was observed with ACTH 1-10, ACTH 11-24, beta-MSH, and beta-Endorphin.

ACTH fragments (ACTH 1-17, ACTH 1-24, ACTH CLIP 18-39, ACTH 22-39, alpha-MSH 1-13) can bind to one of the antibodies and thereby negatively interfere with the sandwich formation and lead to lower ACTH values as shown in the following table:

Cross reactant	Concentration of cross reactant pg/mL	Apparent ACTH pg/mL	Change in ACTH concentration pg/mL	Cross-reactivity %
None; reference	0	44.1	not applicable	not applicable
ACTH 1-17	50000	10.6	-33.5	-0.07
	5000	36.9	-7.2	-0.14
	500	42.6	-1.5	-0.31
ACTH 1-24	50000	10.2	-33.9	-0.07
	5000	37.9	-6.2	-0.12
	500	42.5	-1.6	-0.32
ACTH 18-39	50000	2.0	-42.1	-0.08
	5000	14.9	-29.2	-0.58
	500	37.0	-7.1	-1.42
ACTH 22-39	50000	0.00	-44.1	-0.09
	5000	6.3	-37.8	-0.76
	500	29.4	-14.7	-2.94
alpha-MSH	50000	12.3	-31.8	-0.06
	5000	34.3	-9.8	-0.20
	500	41.3	-2.8	-0.56

Under ACTH 1-24 medication, ACTH measurement is not recommended. POMC (partially purified from an adenoma cell line) showed an approximately 1.6 % cross-reactivity at 1560 pmol/L which is approximately 40 times the physiological concentration of ACTH precursors in circulation.[2]

References

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets 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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

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

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