

REF			SYSTEM
08946728190	08946728500	100	cobas e 402 cobas e 801

## English

### System information

Short name	ACN (application code number)
ACTH	10206

### Intended use

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

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

### Summary

Adrenocorticotrophic 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.<sup>1,2</sup> ACTH stimulates formation and secretion of glucocorticoids (especially cortisol) by the adrenal cortex.

The glucocorticoid production is regulated by various factors.<sup>3,4,5,6</sup> 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.<sup>7,8</sup> 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).<sup>9</sup>

ACTH not produced by the pituitary gland is known as ectopic ACTH;<sup>10</sup> 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.<sup>2</sup>

### Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, a biotinylated monoclonal ACTH-specific antibody, and a monoclonal ACTH-specific antibody labeled with a ruthenium complex<sup>9</sup> 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 II 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 **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)<sub>3</sub><sup>2+</sup>)

### Reagents - working solutions

The **cobas e** pack is labeled as ACTH.

- M Streptavidin-coated microparticles, 1 bottle, 5.8 mL:  
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-ACTH-Ab~biotin, 1 bottle, 7.2 mL:  
Biotinylated monoclonal anti-ACTH antibody (mouse) 0.3 mg/L;  
MES<sup>b)</sup> buffer 50 mmol/L, pH 6.2; preservative.
- R2 Anti-ACTH-Ab~Ru(bpy)<sub>3</sub><sup>2+</sup>, 1 bottle, 7.2 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).

### 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 available via the **cobas** link.

### Storage and stability

Store at 2-8 °C.



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 95<sup>th</sup> percentile value from  $n \geq 60$  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  $\leq 20$  %.

### 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 (5<sup>th</sup>-95<sup>th</sup> 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 402 and cobas e 801 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human plasma 1	3.79	0.170	4.5	0.272	7.2
Human plasma 2	25.9	0.334	1.3	0.746	2.9
Human plasma 3	52.8	0.675	1.3	1.54	2.9
Human plasma 4	886	9.66	1.1	25.1	2.8
Human plasma 5	1919	18.8	1.0	61.9	3.2
PC <sup>c)</sup> Multimarker 1	49.5	0.604	1.2	1.65	3.3
PC Multimarker 2	867	5.78	0.7	27.6	3.2

c) PC = PreciControl

cobas e 402 and cobas e 801 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human plasma 1	0.835	0.037	4.5	0.060	7.2
Human plasma 2	5.70	0.074	1.3	0.164	2.9
Human plasma 3	11.6	0.149	1.3	0.339	2.9
Human plasma 4	195	2.13	1.1	5.53	2.8
Human plasma 5	423	4.14	1.0	13.6	3.2
PC Multimarker 1	10.9	0.133	1.2	0.363	3.3
PC Multimarker 2	191	1.27	0.7	6.08	3.2

### Method comparison

a) A comparison of the Elecsys ACTH assay, [REF] 08946728190 (cobas e 801 analyzer; y), with the Elecsys ACTH assay, [REF] 07026684190 (cobas e 801 analyzer; x), gave the following correlations (pg/mL):

Number of samples measured: 146

Passing/Bablok<sup>11</sup> Linear regression

$$y = 1.02x + 3.46$$

$$y = 1.02x + 2.78$$

$$\tau = 0.972$$

$$r = 1.00$$

The sample concentrations were between 1.68 and 1838 pg/mL.

b) A comparison of the Elecsys ACTH assay, [REF] 08946728190 (cobas e 402 analyzer; y), with the Elecsys ACTH assay, [REF] 08946728190 (cobas e 801 analyzer; x), gave the following correlations (pg/mL):

Number of samples measured: 182

Passing/Bablok<sup>11</sup> Linear regression

$$y = 0.969x - 0.500$$

$$y = 0.936x + 2.39$$

$$\tau = 0.980$$

$$r = 0.998$$

The sample concentrations were between 2.41 and 1983 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, beta-Endorphin and POMC.

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	55.4	not applicable	not applicable
ACTH 1-17	50000	16.9	-38.5	-0.077
	5000	50.9	-4.5	-0.089
	500	54.4	-1.0	-0.203
ACTH 1-24	50000	10.1	-45.3	-0.091
	5000	49.1	-6.3	-0.126
	500	55.3	-0.1	-0.022
ACTH 18-39 (CLIP)	50000	47.8	-7.6	-0.015
	5000	54.7	-0.7	-0.013
	500	55.8	0.4	0.075

Cross-reactant	Concentration of cross-reactant pg/mL	Apparent ACTH pg/mL	Change in ACTH concentration pg/mL	Cross-reactivity %
None; reference	0	55.4	not applicable	not applicable
ACTH 22-39	50000	7.58	-47.8	-0.096
	5000	37.5	-17.9	-0.357
	500	52.9	-2.5	-0.491
ACTH 1-13 (alpha-MSH)	50000	29.2	-26.2	-0.052
	5000	51.4	-4.0	-0.080
	500	55.3	-0.1	-0.022

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Additions, deletions or changes are indicated by a change bar in the margin.

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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](http://dialog.roche.com) for definition of symbols used):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume for reconstitution
	Global Trade Item Number