ACE high sensitive

Procedure KK-ACF

A Commitment to Diagnostics

Pre-Analytics

Samples required:	~250 µl CSF
	~100 µl Serum 1:5 in NaCl
	(optionally, Heparin plasma can be used; EDTA plasma inhibits ACE activity)
Sample collection:	CSF collection by lumbar puncture
	Serum in collection tubes without anti-coagulants
Sample storage:	at 2-8°C up to 24 h at -20°C for longer storage

Special Equipment

Manual procedure:
Microtiterplate reader with a filter at 340 nm, incubation chamber at 37°C and software suitable for kinetic measurements.

Microtiterplates, e.g. NUNC Maxisorb F8

Number of Tests

	KK-ACF
Tests	130
Substrate	1 x 11 ml
Calibrator	1 x 2 ml
Controls normal/high	1 x 2 ml each

Manual procedure (in duplicates)

Reagents have to be adjusted to 18 - 28 °C .

Dilute the Calibrator 1:5 in NaCl
Dilute serum samples 1:5 in NaCl (optional)

Pipett 80 µl Calibrator (1:1) and 80 µl Calibrator (1:5) into the wells B2, B3 and C2 to C3, respectively (two-point calibration).

Pipett 80 µl Control low and high into the wells D2, D3 and E2 to E3, respectively.

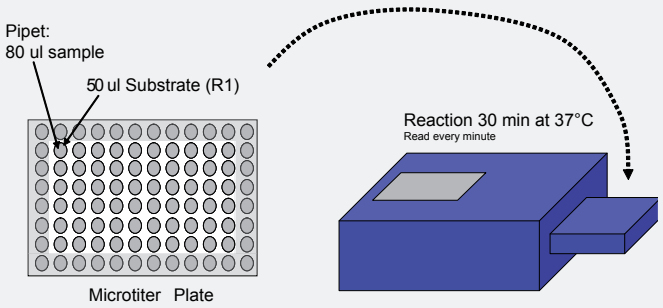
Pipett 80 µl CSF (1:1) or diluted serum (1:5) into the subsequent wells.

Pipett 50 µl Substrate into each well.

incubate for 30 min at 37°C and record the OD at 340 nm at intervals of exactly 1 min.

It is important to maintain a lag time of three minutes. Thus, the reading points up to t=180 sec. (lack-time) have to be excluded from the calculation.

Calculate the Δ OD(Units/min) at the reading points between 180 and 1800 sec and calculate the results for controls and samples from the standard curve.



Automated Procedure General Settings

Open clinical chemistry analysers with incubation times >15 min and syringes able to dispense up to 80 µl sample.

	Setting
Test type	photometric
Result unit	U/L (ACE units/L)
Sample type	CSF or diluted serum sample
Calibration type	linear
Kinetic reaction	descending
Calibration	2-point
Substrate (R1)	80 µl
Incubation	90 sec
Sample	80 µl
Incubation	120 sec.
Measurement	kinetic
Wavelength	340 nm
Measuring time	900 sec.

BÜHLMANN

ACE high sensitive

Characteristics KK-ACF

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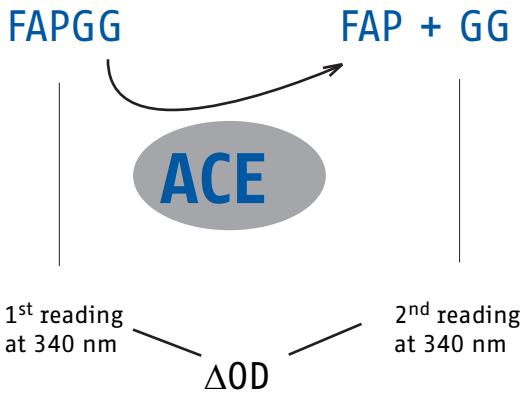
Intended Use

Direct and quantitative determination of angiotensin converting enzyme (ACE) activity in cerebrospinal fluid (CSF) and diluted serum samples by an enzymatic assay.

Principle of the Assay

In vivo, Angiotensin Converting Enzyme (ACE) catalyses the conversion of angiotensin I to angio-tensin II. In vitro, the enzyme also mediates the cleavage of the synthetic substrate (FAPGG =N-[3-(2-furyl)acryloyl]-L-phenylalanyl-L-glycyl-L-glycine) into an amino acid derivate and a dipeptide. The kinetic of this cleavage reaction is measured by recording the decrease in absorbance at 340 nm.



Assay Performance Data

Data have been established with the manual procedure on microtiterplates.

Repeatability <11 %

Total precision <20 %

CSF samples and spiked NaCl solution.
n=5 samples tested over a period of 10 days

Dilution linearity 1-24 U/L

The assay is linear between 1-24 U/L.

Spiking recovery 97-119 %

4 samples spiked with increasing anmounts of ACE.

Sensitivity

LoB: 0.3 ACE U/L
LoD: 1.0 ACE U/L
LoQ: < 1.5 ACE U/L

Specificity

The ACE activity can be dose-dependently inhibited by its natural substrate Angiotensin I, by the chelator EDTA, and by H-Val-Trp-OH.

Normal Values

Patients without neurosarcoidose (CSF)	
n	178
Mean (U/l)	0.3
95th Percentile (U/l)	1.3
Max. value (U/l)	1.9
Proposed Cut-off (U/l)	2.0

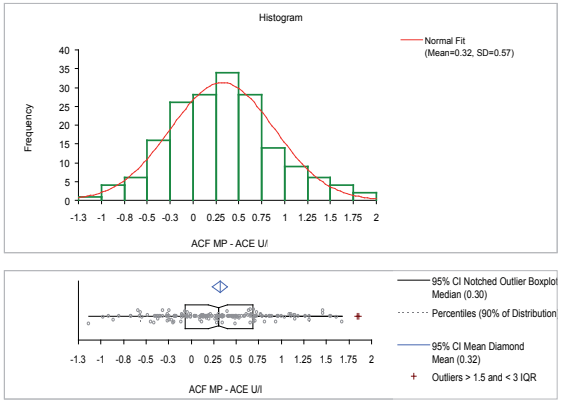


Figure 1: Frequency distribution of normal values in CSF

Method comparison

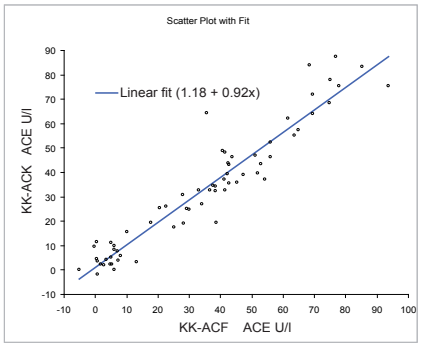


Figure 2: Method comparison with CSF and diluted serum samples

Ordering code:

KK-ACF ca.130 tests
KK-ACK 100 tests

CE-marked products

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