ACE high sensitive

Angiotensin Converting Enzyme in Cerebrospinal Fluid (CSF)

This product is for research use only
It is not intended for use in diagnostic procedures

KK-ACF-U

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ENGLISH

INTENDED USE

The BÜHLMANN ACE high sensitive test (KK-ACF) is intended for the direct and quantitative determination of angiotensin converting enzyme (ACE) activity in cerebrospinal fluid (CSF) and in diluted serum samples by an enzymatic assay. This product is for research use only. It is not intended for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

ACE catalyses the conversion of angiotensin I to angiotensin II. The enzyme also mediates the cleavage of the synthetic substrate (FAPGG) into an amino acid derivative and a dipeptide. The kinetic of this cleavage reaction is measured by recording the decrease in absorbance at 340 nm.

REAGENTS SUPPLIED AND PREPARATION

Reagent	Quantity	Code	Reconstitution
Substrate	1 vial 11 ml	B-ACF-SUB	Ready to use
Calibrator[1]	1 vial lyophilized	B-ACF-CA	Add 2 ml of deionized water
Controls[2] Low and high	2x 1 vial lyophilized	B-ACF-CONSET	Add 2 ml of deionized water

Table 1

[1] Lyophilized ACE Calibrator in a protein buffer matrix with lot specific activity. After reconstitution leave for 15 minutes at 18-28°C and mix well before use.

[2] Lyophilized ACE low and high Controls in a protein buffer matrix with lot specific activity. Reconstitute for 15 minutes at 18-28°C and mix well before use.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
Stable at 2-8°C until expiration date printed on the label	
Opened / Reconstituted Reagents	
Substrate	Stable until exp. date at 2-8°C
Calibrator	Stable for 6 months at 2-8°C
Controls	

Table 2

WARNINGS AND PRECAUTIONS

The calibrator and controls of this kit contain components of human origin. Each serum used to prepare the kit components was tested by an FDA approved method and found negative for HBV surface antigen, as well as for HCV and HIV1/2 antibodies. Although these methods are highly accurate, there is no guarantee that this material cannot transmit Hepatitis or AIDS. *Therefore, all specimens and kit components should be handled as potentially infectious.* All products containing human source material should be handled according to good laboratory practice using appropriate precautions.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipetes: 100 µl, 1000 µl
- Sterile physiological NaCl solution (0.9%) for Calibrator and serum sample dilution
- Microtiter plate
  e.g. Maxisorp F8, NUNC Corp. (hand method)
- Microtiter plate reader with kinetic function; 37°C incubation chamber (hand method)
- Manual application: If you use a plate reader without incubator, an external 37°C microtiter plate incubator is needed e.g. Eppendorf Thermomixer comfort.
- Automated application: Clinical chemistry analyzers with incubation time option of 15 min and sample volume option of ~80 µl.

SPECIMEN COLLECTION AND STORAGE

Collect at least 250 µl CSF by lumbar puncture and store it at 2-8°C for up to 24 h or at -20°C for longer storage.

ASSAY PROCEDURE

Hand Method

Make sure that the chamber of your Microtiter plate reader can be adjusted to 37°C – kinetic reader. Program the reader for a 30 min kinetic measurement at 340 nm; measuring points at every minute.

Allow the reagents and samples to equilibrate to 18-28°C prior to use.

CSF sample have to be used undiluted

1. Prepare a 1:5 dilution of the Calibrator with 0.9% NaCl solution (e.g. 60 µl Calibrator and 240 µl NaCl solution).
2. **Optional:** If testing serum samples in this assay prepare 1:5 dilutions of the serum samples with 0.9% NaCl solution (e.g. 60 µl serum and 240 µl NaCl solution).

In order to avoid temperature effects within the microplate leave the first and last strip of the test and the first and last well each strip empty.

We recommend carrying out the test **in duplicates**.

3. Pipet 80 µl Calibrator (undiluted) in duplicates into wells B2 and B3.
4. Pipet 80 µl Calibrator (1:5 diluted) in duplicates into wells C2 and C3.
5. Pipet 80 µl Control low and high in duplicates into wells D2, D3 and E2, E3, respectively.
6. Pipet 80 µl CSF samples (and/or diluted serum samples) in duplicates into the following wells.
7. Pipet 50 µl Substrate to each reaction well.
8. Shake the plate gently and start reading at 340 nm for 30 min.

Note: Maintaining a lag time of around 3 minutes is important. Thus disregard the measuring points 1-3 for calculation (pre-incubation time).

DATA REDUCTION

Calculate the slope (Vmax = milli units/min) of the Calibrators, Controls and samples from time 180 s to 1800 s. Create a calibration curve with the two calibrators and calculate the results of controls and samples.

Definition: One unit of ACE activity is defined as the amount of enzyme required to release one µmol of hippuric acid per minute and per liter of serum at 37°C:

$$1\,\text{ACE unit} = \frac{1\,\mu\text{mol hippuric acid}}{\text{min} \times L} = 1\,U/l$$

Refer to Table 4 and Figure 1 for typical data. *These results and standard curve are provided for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.*

APPLICATION ON CLINICAL CHEMISTRY ANALYZERS

The test can be automated on any clinical chemistry analyzer allowing an incubation time of >15 min and a sample volume of 80 µl.

General instrument settings

Test type	photometric
Result unit	U/l (ACE units per liter)
Sample type	CSF
Calibration type	linear
Curve direction	descending
Calibration	duplicates
Substrate (R1)	80 µl
Incubation	90 sec
Sample	80 µl
Incubation	120 sec
Measurement	kinetic
Wavelength	340 nm
Measuring Time	900 sec

QUALITY CONTROL

The values of the internal low and high Controls provided with the kit must be within the lot specific range indicated on the corresponding data sheet. Otherwise, the assay has to be repeated.

It is good laboratory practice to record the following data for each assay: kit lot number, reconstitution dates of kit components, concentration value of calibrator and controls, concentration values of internal pool sample.

STANDARDIZATION

The BÜHLMANN ACE high sensitive assay standardization is based on the BÜHLMANN ACE kinetic assay (KK-ACK).

PERFORMANCE CHARACTERISTICS

The performance data has been established with the hand method on microtiter plates.

Detection Limits

Limit of Blank (LoB): 0.3 ACE U/L. The LoB has been established in two independent runs with a total of 84 blank values with 0.9% NaCl-solution. The LoB is calculated as mean + 2SD.

Limit of Detection (LoD): 1.0 ACE U/L. The LoD has been established with four independent CSF samples spiked with ACE at concentrations between 0.5 and 1.5 U/L. Spiked samples were measured in the same run 20 times. The LoD has been calculated in accordance with CLSI protocol EP17-A.

Limit of Quantification (LoQ): <1.5 ACE U/L. The LoQ has been established with NaCl-solution spiked with ACE at concentrations between 0.5 and 3.0 U/L. Spiked samples were tested 10 times in two independent runs, each. A limit of 20% CV was applied.

Linearity: 1.0-24 ACE U/L. Serial dilution of two independent samples showed linearity within the indicated range (cf. Figure 2).

Recovery: 97-119%. Four independent CSF samples were spiked with increasing concentration of ACE and measured according to the assay procedure. Good recovery was found within the linear range of the assay (cf. Table 5).

Precision: Repeatability: <11% CV; Total precision <20% CV. CSF samples and $NaCl^-$-solution each spiked with ACE where tested over a period of 10 days. Each sample was run in duplicate and independently tested twice per day (cf. Table 6)

METHOD COMPARISON

CSF and Serum samples were used for correlation between application and methods. Using the ACE high sensitive assay as the reference method, the following correlations where found:

Correlation	n	R^2	Bias	Slope
ACF vs. ACF Konelab	132	0.97	-0.11	1.284
ACF vs. ACF Cobas Mira	110	0.95	-0.35	1.058
ACF vs. ACD*	59	0.96	1.43	0.888
ACF vs. ACK	86	0.91	1.176	0.921

Table 3

* cf. see Figure 3

ACF: ACE high sensitive hand method
ACF Konelab: ACE high sensitive application on KoneLab T30
ACF Cobas Mira: ACE high sensitive application on Cobas Mira
ACD: ACE direct (RK-ACD) sensitive radio-enzymatic assay
ACK: ACE kinetic (KK-ACK) reference method for serum samples.

Table 4 **Example of Results**

Sample	Wells	Rate	Result	Mean Result	SD	CV%
Cal1	C2 C3	-2.013 -1.959	10.963 10.637	10.8	0.163	1.5
Cal2	D2 D3	-0.561 -0.566	2.145 2.175	2.16	0.015	0.7
con high	E3 E4	-1.051 -1.041	5.118 5.061	5.1	0.0285	0.6
con low	E2	-0.221	0.076	0.1		
S1	F2 F3	-0.299 -0.227	0.55 0.112	0.3	0.219	66.2
S2	G2 G3	-0.437 -0.401	1.392 1.173	1.3	0.1095	8.8
S3	B5 B6	-0.458 -0.478	1.518 1.64	1.6	0.061	3.8
S4	C5 C6	-0.837 -0.863	3.818 3.977	3.9	0.0795	2.0
S5	D5 D6	-1.617 -1.603	8.562 8.473	8.5	0.0445	0.5
S6	E5 E6	-2.367 -2.383	13.115 13.211	13.2	0.048	0.4
S7	F5 F6	-4.01 -4.009	23.097 23.091	23.0	0.003	0.0

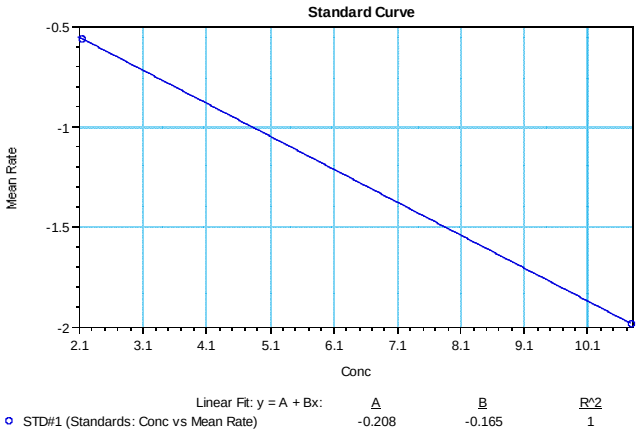
Table 5 **Recovery**

Spiked With [U/L]	S1 Obs [U/L]	S1 O/E [%]	S2 Obs [U/L]	S2 O/E [%]	S3 Obs [U/L]	S3 O/E [%]	S4 Obs [U/L]	S4 O/E [%]
1.5	1.29	86.2	1.80	120.3	2.30	153.6	1.63	108.5
2	1.64	81.8	2.63	131.4	2.70	134.8	2.30	114.8
4	4.00	100.0	4.80	120.0	4.69	117.1	5.94	148.5
8	8.56	107.0	8.75	109.4	8.65	108.1	8.71	108.9
12	13.29	110.7	12.35	102.9	12.81	106.8	12.11	100.9
24	23.20	96.7	22.21	92.5	22.21	92.5	22.34	93.1
Mean		**97.1**		**112.7**		**118.8**		**112.4**

Table 6 **Precision**

Sample	U/L	Repeatability (Within Run)	Between Run	Between Day	Total Precision
S1	2.1	8.6%	11.7%	9.9%	17.5%
S2	4.1	8.0%	4.0%	7.3%	11.5%
S3	5.3	10.4%	5.9%	13.8%	18.3%
S4	10.6	5.8%	4.7%	4.4%	8.6%
S5	19.7	1.3%	3.7%	1.7%	4.3%

Figure 1 **Example of Calibration**



n	178	(cases excluded: 3 due to missing values)			
Mean	0.32		Median	0.30	
95% CI	0.24	to 0.41	95.7% CI	0.19	to 0.40
SE	0.043				
			Range	3.0	
Variance	0.32		IQR	0.76	
SD	0.57				
95% CI	0.51	to 0.63	Percentile		
			0th	-1.13	(minimum)
CV	176.4%		5th	-0.57	
			25th	-0.07	(1st quartile)
Skewness	0.22		50th	0.30	(median)
Kurtosis	0.09		75th	0.69	(3rd quartile)
			95th	1.31	
Shapiro-Wilk W	0.99		100th	1.86	(maximum)
p	0.590				

Figure 2 **Linearity**

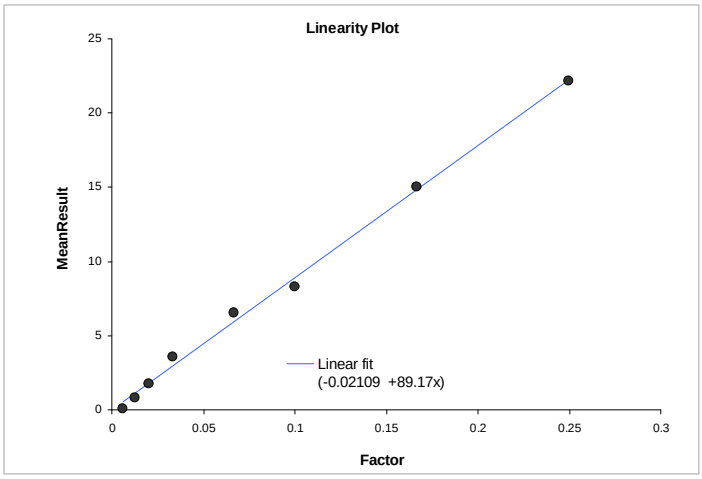
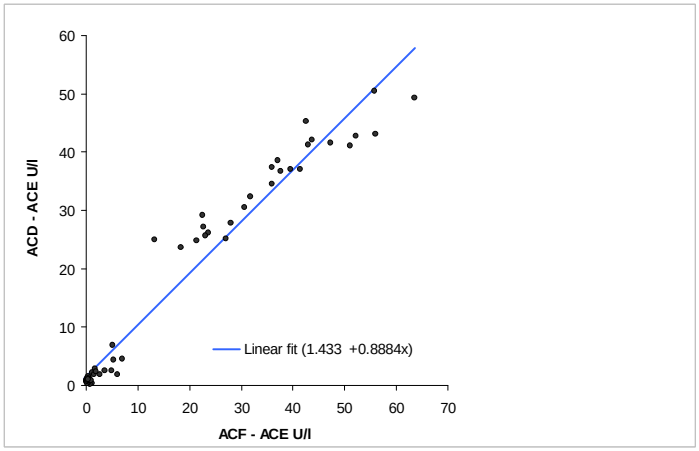


Figure 3 **Correlation with ACE Direct assay**



We would like to thank Dr. Jocelyne Drai, CHU Lyon Sud, France for her important work with the optimization of substrate and buffer system in order to improve the sensitivity of the assay. These changes allow the reliable measurement of ACE activity in cerebrospinal fluid.

APPENDIX I
SYMBOLS

Symbol	Explanation
	Use By Verwendbar bis Utiliser jusqu'au Utilizzare entro Fecha de caducidad
REF	Catalogue number Bestellnummer Référence du catalogue Numero di catalogo Número de catálogo
LOT	Batch code Chargenbezeichnung Code du lot Codice del lotto Codigo de lote
	Temperature limitation Zulässiger Temperaturbereich Limites de température Limiti di temperatura Límites de temperatura
	Consult Instructions for Use- Gebrauchsanweisung beachten Consulter le mode d'emploi Consultare le istruzioni per l'uso Consulte las instrucciones de uso

Symbol	Explanation
	Contains sufficient for <n> tests Ausreichend für „n" Ansätze Contenu suffisant pour „n" tests Contenuto sufficiente per „n" saggi Contenido suficiente para <n> ensayos
CONTROL L	Low Control Kontrolle niedrig Contrôle bas Controllo basso Control bajo
CONTROL H	High Control Kontrolle hoch Contrôle Elevé Controllo alto Control Alto
CAL	Calibrator Kalibrator Calibrateur Calibratore Calibrador
SUBS	Substrate Substrat Substrat Substrato Substrato