BÜHLMANN ACE kinetic

Angiotensin Converting Enzyme

For *In Vitro* Diagnostic Use

Rx Only

KK-ACK	26 mL substrate
KK-ACK2	2 x 13 mL substrate
KK-ACK4	4 x 26 mL substrate
KK-ACKX	3 x 100 mL substrate

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ENGLISH

INTENDED USE

BÜHLMANN ACE kinetic is an *in vitro* diagnostic biochemical assay for the quantitative determination of angiotensin converting enzyme (ACE) activity in serum samples. The assay aids the assessment of disease activity in patients with sarcoidosis in conjunction with other clinical and laboratory findings.

For laboratory use only.

PRINCIPLE OF THE ASSAY

The assay is a quantitative enzymatic test which can be easily applied on clinical chemistry analyzers or run by manual method. ACE catalyzes the conversion of angiotensin I to angiotensin II. The enzyme also mediates the cleavage of the synthetic substrate Furylacryloylphenylalanylglycylglycine (FAPGG) into the amino acid derivative Fyrylacryloylphenylalanine (FAP) and the dipeptide Glycylglycine (GG). The linear kinetic of this cleavage reaction is measured by recording the decrease in absorbance at 340 nm (ref. 1, 2). The final ACE activity in U/L in the patient sample is determined using a calibration curve generated from the measured calibrator value.

REAGENTS SUPPLIED

Reagents	Quantity			Code	Recon-stitution
	KK-ACK/ KK-ACK4	KK-ACK2	KK-ACKX		
Substrate	1 vial/ 4 vials 26 mL	2 vials 13 mL	3 vials 100 mL	B-ACK-SUB B-ACK2-SUB[1] B-ACKX-SUB[2]	Ready to use
Calibrator[3]	1 vial/ 2 vials	2 vials	3 vials	B-ACK-CA	Add 2 mL of deionized water
Controls[4] Normal and High	1x2 vials/ 2x2 vials	2x2 vials	3x2 vials	B-ACK-CONSET	Add 2 mL of deionized water

Table 1

1 Order Codes for KK-ACK2.
2 Order Codes for KK-ACKX.
3 Lyophilized ACE Calibrator in a protein serum matrix with lot specific activity. After reconstitution leave for 15 minutes at 18-28 °C and mix well before use.
4 Lyophilized ACE Normal and High Controls in a protein serum matrix with lot specific activity. Reconstitute for 15 minutes at 18-28 °C and mix well before use.

STORAGE AND STABILITY OF REAGENTS AND WORKING SOLUTIONS

Unopened reagents	
Store at 2-8 °C. Do not use the kit past expiration date printed on the labels.	
Opened / reconstituted reagents	
Substrate	Store for up to 6 months at 2-8 °C.
Calibrator	
Controls	
On-board stability on clinical chemistry analyzers	
Store for up to 73 days at temperatures ≤ 15°C.	

Table 2

MATERIALS REQUIRED BUT NOT PROVIDED

- Clinical chemistry analyzer with 340 nm filter.
- Manual method only:
  - Waterbath set at 37 °C
  - Precision pipettes for 25 µL, 100 µL and 1 mL
  - Spectrophotometer with incubation at 37 °C

WARNINGS AND PRECAUTIONS

- This test is for *in vitro* diagnostic use only.
- It is recommended that the test be handled by qualified personnel, in accordance with Good Laboratory Practice (GLP).

Safety precautions

- Unused solution should be disposed of according to local state and federal regulations.

Technical precautions

- Read the instructions carefully prior to carrying out the test. Test performance will be adversely affected, if reagents are incorrectly diluted, handled or stored under conditions other than those detailed in this instruction for use.
- Components must not be used after the expiry date printed on the labels.
- Do not mix different lots of reagents.
- Ensure that samples have no bubbles prior to running the test.
- Please equilibrate reagents, controls, calibrators and samples at analyzer storage temperature or for manual use at room temperature. Reconstitute the lyophilized reagents as indicated. Mix the reconstituted reagents well before use.
- Avoid evaporation of the controls.

SPECIMEN COLLECTION AND STORAGE

Collect blood into standard serum collection tubes and avoid hemolysis. To avoid lipemic sera, collect blood from fasting patients. Prepare serum according to your laboratory's standard procedure. Serum preparation may also be performed using gel separator tubes (SST).

A minimum of 200 µL serum sample for the test is recommended. For the exact volume, refer to the instrument application note.

Storage: Serum samples can be stored unrefrigerated (temperatures up to 28 °C) or at 2-8 °C for 10 days. For longer storage keep samples at ≤-20 °C. The samples are stable for at least 7 months at ≤-20 °C. More than four (4) freeze/thaw cycles should be avoided.

ASSAY PROCEDURE

Application notes / assay installation

Assay procedures for the BÜHLMANN ACE kinetic are established on several clinical chemistry analyzers. Validated application notes describing installation and analysis on specific instruments are available from BÜHLMANN upon request. Corresponding instrument manuals must be considered for instrument setup, maintenance, operation and precautions.

BÜHLMANN ACE kinetic can also be used with a manual method. The procedure description is made available from BÜHLMANN upon request.

Reagent preparation

The substrate is ready to use. Transfer the necessary volume to the analyzer specific bottles/cassettes.

Establishment of the calibration curve

The calibrator included in the kit is used to establish a two-point calibration curve according to the instrument manual. Calibrator values are lot-specific. A new calibration must be performed for each new lot. Otherwise, periodic calibrations should be performed according to the instrument specific application notes. Refer to the QC-data sheet provided with the BÜHLMANN ACE kinetic kit for assigned calibrator values. Contact BÜHLMANN support if calibration cannot be performed without error.

QC controls

The controls included should be assayed each day before running patient samples to validate the calibration curve. The control measurements must be within the value ranges, indicated in the QC-data sheet, to obtain valid results for patient samples. If the control values are not valid, repeat measurement with fresh controls. If control values remain invalid, recalibrate the assay. If valid control values cannot be reproduced, after performing the steps described above, contact BÜHLMANN support.

Results

Results are calculated automatically on the clinical chemistry analyzer and are presented in U/L.

STANDARDIZATION

- The BÜHLMANN ACE kinetic calibrator is used to generate a two-point calibration curve. Calibrator values are assigned using a UV/VIS spectrophotometric method and a defined $\Delta\varepsilon$ of the FAPGG substrate. The values are reported in the QC data sheet. The calibrator material comprises ACE from rodent serum in a buffer matrix. The 95% confidence interval of the combined uncertainty of the product calibrator is lower than 5.0%.
- The analytical measuring interval of the ACE kinetic assay, established on the Roche cobas® 6000 c501 instrument, is 11.3 - 200 U/L and can be further extended to 500 U/L, using automatic re-run programs available on clinical chemistry analyzers.

LIMITATIONS

- Test results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.
- The serum ACE activity strongly depends on the genotype of the patients investigated (ref. 3). Results should be reviewed in the context of previous BÜHLMANN ACE kinetic results obtained for the patient.
- ACE activity testing should not be performed in patients treated for hypertension with ACE inhibitors such as Benazepril (Lotensin), Captopril, Enalapril (Vasotec). No interference has been detected for antihypertensive drugs containing angiotensin II receptor blockers (AT1-antagonists): Losartan and Eprosartan.
- Hemolysis, icterus and lipemia interfere with the assay. Refer to section “Interfering Substances” for serum indices.
- BÜHLMANN ACE kinetic is validated for serum samples only. It is the decision of the laboratory whether samples incorrectly collected into lithium-heparin or citrate tubes should be processed (please refer to section plasma samples for more information).
- EDTA is an inhibitor of ACE activity. EDTA plasma cannot be used with the BÜHLMANN ACE kinetic assay.

REFERENCE INTERVALS

Adults: The following reference interval was established for BÜHLMANN ACE kinetic based on 2.5^{th} - 97.5^{th} percentile values obtained for healthy participants enrolled in three independent studies in Switzerland (n=80, age: 20 - 70), Germany (n=159, age: 18 - 64, ref. 3) and USA (n= 327, age 16 - 77, ref. 4):

20 – 70 U/L

Children: The following reference interval was established for BÜHLMANN ACE kinetic based on 2.5^{th} - 97.5^{th} percentile values obtained for healthy pediatric participants enrolled in a single study in Germany (n=84, age: 0.5 - 18):

33 – 112 U/L

PERFORMANCE CHARACTERISTICS

The presented performance characteristics have been established on a Roche cobas® 6000 c501 instrument, unless otherwise indicated. Refer to clinical chemistry analyzer specific application notes for the performance characteristics on other clinical chemistry analyzers.

Reproducibility: 6.3 - 9.1% CV

Reproducibility was established according to the CLSI guideline EP05-A3 using a 3 instruments/lots x 5 days x 5 replicates study design. An acceptance criterion of 15% CV and 20% CV, for samples above and below 40 U/L, respectively, was applied. Testing was performed at 2 laboratory sites, using Roche c501, Roche c702 and Beckmann Coulter AU instruments. Six (6) serum samples were tested. The results are presented in table 3.

Repeatability: 0.8 - 3.0% CV

Within-Laboratory precision: 1.7 - 3.7% CV

Repeatability and within-laboratory precision were established according to the CLSI guideline EP05-A3 using the standardized 20 days x 2 runs x 2 replicates study design. An acceptance criterion of 10% CV and 15% CV, for repeatability and within-laboratory precision, respectively, for samples above 40 U/L, was applied. For samples below 40 U/L the acceptance criterion was 20% CV. Six (6) serum samples were tested. The results are presented in table 4.

Accuracy / Recovery: 92.0 - 112.8%

Six (6) serum samples with ACE activity values covering the BÜHLMANN ACE kinetic measuring range were spiked with 20.5 U/L ACE obtained from calibrator

material. Spiking was performed at 10% of the sample volume. “Baseline” samples were spiked with the corresponding volume of 0.9% NaCl. “Baseline” and “baseline + spike” samples were measured in four (4) replicates. The results are summarized in table 5.

Sample carry-over

The sample carry-over was established according to the CLSI guideline EP10-A2. No statistically significant carry-over with the ACE kinetic test on Roche cobas® 6000 c501 instrument was detected.

Limit of Detection (LoD): 6.8 U/L

The LoD was established according to the CLSI guideline EP17-A2 using the classical approach, parametric analysis and a LoB of 4.3 U/L, determined using a non-parametric analysis.

Limit of Quantification (LoQ): 11.3 U/L

The LoQ was established according to the CLSI guideline EP17-A2, based on 60 determinations and a precision goal of 20% CV.

Linearity range: 4.3 - 534.9 U/L

The linear range of the BÜHLMANN ACE kinetic was determined according to the CLSI guideline EP06-A. Samples with an activity over 150 U/L were automatically re-run using a reduced sample volume. A maximum deviation from linearity of ±4 U/L or ±10% was allowed.

Security Zone

Samples with theoretical ACE activity of up to 541.2 U/L can be measured without limiting the measuring range of the assay.

INTERFERING SUBSTANCES

The susceptibility of the BÜHLMANN ACE kinetic assay to interfering substances was assessed according to the CLSI guideline EP07-A2. Bias in results exceeding 20% was considered interference.

Oral pharmaceuticals

No interference was detected with the following substances; Aspirin (0.65 mg/mL), Azathioprine (3.0 µg/mL), Chlorambucil (7.2 µg/mL), Cyclophosphamide (0.375 mg/mL), Eprosartan (0.36 mg/mL), Hydroxychloroquine (up to 0.06 mg/mL), Ibuprofen (0.5 mg/mL), Losartan (0.09 mg/mL), Methotrexate (2.0 µg/mL), Prednisone (0.3 µg/mL).

Serum indices

Interference was detected with the following substances at the listed concentrations: triglycerides (2.24 mg/mL), conjugated bilirubin (0.06 mg/mL), unconjugated bilirubin (0.047 mg/mL), and hemoglobin (1.19 mg/mL). No interference by triglycerides was observed when samples with turbidity were subjected to short centrifugation (10 min / 12’000 x g) and separation of lipid-containing supernatant.

Plasma samples

Results from samples from healthy blood donors collected into lithium-heparin and citrate tubes were compared to results obtained with serum samples from the same donors, collected according to the instruction for use. Bias was determined using Passing-Bablok linear regression and Bland-Altman analysis. The results are summarized in table 6.

TABLES AND FIGURES

Reproducibility

ID	Mean ACE activity [U/L]	n	Within-run		Between-day		Between-lot/ instrument		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
12400	29.6	75	1.9	6.3%	0.0	0.0%	2.0	6.6%	2.7	9.1%
12401	46.9	75	1.5	3.3%	0.0	0.0%	3.3	7.1%	3.7	7.8%
12402	73.5	75	2.2	3.0%	0.0	0.0%	4.5	6.1%	5.0	6.8%
12403	121.5	75	4.0	3.3%	2.5	2.0%	6.1	5.0%	7.7	6.3%
12404	217.2	75	6.7	3.1%	5.6	2.6%	13.0	6.0%	15.7	7.2%
12405	310.3	75	13.6	4.4%	11.9	3.8%	11.4	3.7%	21.3	6.9%

Table 3

Within-laboratory precision

ID	Mean ACE activity [U/L]	n	Within-run		Between-run		Between-day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
12850	28.2	80	0.9	3.0%	0.5	1.6%	0.4	1.3%	1.0	3.7%
12851	44.2	80	1.0	2.3%	0.3	0.8%	0.3	0.7%	1.1	2.5%
12852	68.5	80	1.0	1.5%	0.8	1.2%	0.7	1.0%	1.5	2.1%
12853	119.4	80	1.0	0.8%	1.4	1.1%	1.2	1.0%	2.0	1.7%
12854	213.9	80	3.0	1.4%	3.4	1.6%	2.3	1.1%	5.1	2.4%
12855	364.8	80	3.7	1.0%	5.2	1.4%	3.8	1.1%	7.4	2.0%

Table 4

Recovery

Sample ID	12615	12618	12614	12558	3198532	3190624
Base value [U/L]	19.8	34.6	59.0	69.8	75.8	104.6
Spike value [U/L]	20.5	20.5	20.5	20.5	20.5	20.5
Expected value [U/L]	40.3	55.1	79.5	90.3	96.3	125.1
Observed value [U/L]	37.1	56.9	80.8	89.8	108.7	130.0
Total recovery [%]	92.0	103.3	101.7	99.4	112.8	103.9

Table 5

Plasma samples

Matrix	N	Bland-Altman Analysis			Passing-Bablok Regression Analysis		
		Mean bias (95 % CI)	Upper LoA (95 % CI)	Lower LoA (95 % CI)	Slope (95 % CI)	Intercept (95 % CI)	r
Lithium-heparin plasma	38	-1.1% (-4.5 to 2.3)	20.7% (14.9 to 26.6)	-23.0% (-28.8 to -17.1)	0.9 (0.8 to 1.0)	2.5 (0.2 to 5.4)	0.975
Citrate plasma	44	-10.8% (-13.9 to -7.6)	8.1% (2.6 to 13.5)	-29.6% (-35.0 to -24.2)	0.8 (0.8 to 0.9)	1.7 (-0.7 to 4.4)	0.990

Table 6

REFERENCES

1. Ronca-Testoni S.: Direct spectrophotometric assay for angiotensin-converting enzyme in serum. Clin Chem 29, 1093-1096 (1983).
2. Bénéteau B. and Baudin B. et al.: Automated kinetic assay of angiotensin-converting enzyme in serum. Clin Chem 32, 884-886 (1986).
3. Biller H, Zissel G, Müller-Quernheim J et al.: Genotype-corrected reference values for serum angiotensin-converting enzyme. Eur Respir J 28, 1085-90 (2006).
4. Chen, S. X., Hermelin, D. & Weintraub, S. J. Possible donor-dependent differences in efficacy of fresh frozen plasma for treatment of ACE inhibitor–induced angioedema. *J. Allergy Clin. Immunol. Pract.* **7**, 2087–2088 (2019).

CHANGELOG

Date	Version	Change
2023-07-04	A1.1	Revision of chapter *symbols*

INCIDENT REPORTING IN EU MEMBER STATES

If any serious incident in relation to this device has occurred, please report without delay to the manufacturer and competent authority of your Member State.

SHIPPING DAMAGE

Please notify your distributor, if this product was received

damaged

SYMBOLS

BÜHLMANN use symbols and signs listed and described in ISO 15223-1. For definition of symbols see the symbol glossary at: www.buhlmannlabs.ch/support/downloads/

In addition the following symbols and signs are used:

Symbol	Explanation
Control N	Control Normal
Control H	Control High
CAL	Calibrator
SUBS	Substrate
	**EN**: electronic instruction for use available in different languages at:/ **BG**: електронни инструкции за употреба на различни езици на адрес:/ **CS**: elektronický návod k použití dostupný v různých jazycích na adrese:/ **DA**: elektronisk brugsanvisning på forskellige sprog på:/ **DE**: elektronische Gebrauchsanweisung in verschiedenen Sprachen verfügbar unter:/ **EL**: ηλεκτρονικές οδηγίες χρήσης διαθέσιμες σε διάφορες γλώσσες στη διεύθυνση:/ **ES**: instrucciones de uso electrónicas disponibles en diferentes idiomas en:/ **ET**: elektrooniline kasutusjuhend, mis on saadaval erinevates keeltes aadressil:/ **FR**: un mode d'emploi électronique disponible en différentes langues à l'adresse:/ **HU**: különböző nyelveken elérhető elektronikus használati utasítás a következő címen:/ **IT**: istruzioni elettroniche per l'uso disponibili in diverse lingue su:/ **LT**: elektroninės naudojimo instrukcijos įvairiomis kalbomis:/ **LV**: dažādās valodās pieejama elektroniska lietošanas instrukcija:/ **NO**: elektronisk instruksjon for bruk tilgjengelig på forskjellige språk på:/ **PL**: elektroniczna instrukcja obsługi dostępna w różnych językach na stronie:/ **PT**: instrução electrónica para utilização disponível em diferentes línguas em:/ **RO**: instrucțiuni electronice de utilizare disponibile în diferite limbi la adresa:/ **SK**: elektronický návod na použitie dostupný v rôznych jazykoch na:/ **SL**: elektronska navodila za uporabo so na voljo v različnih jezikih na:/ **SR**: elektronsko uputstvo za upotrebu dostupno na različitim jezicima na:/ **SV**: elektronisk bruksanvisning på olika språk på följande adress: **www.buhlmannlabs.ch/support/downloads/**

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