ACE FS*

Order Information

Cat. No.	Kit size				
1 7601 99 10 930	R1	4 x 20 mL	+	R2	2 x 10 mL

Intended Use

Diagnostic reagent for quantitative in vitro determination of angiotensin-converting enzyme (ACE) activity in human serum or lithium heparin plasma on automated BioMajesty® JCA-BM6010/C.

ACE FS is intended to be used in conjunction with other tests like 1,25-dihydroxyvitamin D, calcium and alkaline phosphatase to confirm the diagnosis of sarcoidosis, for long-term assessment of clinically active sarcoidosis, and to monitor the efficacy of initiated drug therapy [1,2].

Summary

Angiotensin-converting enzyme (ACE) is a zinc metallopeptidase with a molecular weight of 150–180 kDa. ACE is a central component of the renin-angiotensin-aldosterone system (RAAS) and plays a crucial role in circulatory homeostasis by causing blood vessel constriction through the conversion of angiotensin I to angiotensin II. The metallopeptidase is not substrate-specific and cleaves various other peptides, including bradykinin that causes vasodilation and increased vascular permeability, leading to inflammation [3]. ACE is localized on the luminal side of the vascular endothelium of most organs including kidney, heart, brain, and skeletal muscle, as it is a part of local RAASs, while the highest ACE activities are found in the endothelial cells of the pulmonary capillaries [1].

Since the end of the 20th century, it is well known that the level of circulating ACE depends on an insertion/deletion (I/D) polymorphism. Three genotypes exist: DD, ID, and II, with ACE levels being highest in DD, intermediate in ID, and lowest in II [4,5]. The distribution of the genotypes is in accordance with the Hardy–Weinberg equilibrium; II, ID, and DD genotypes are represented in the population at approximately 20%, 43% and 37% respectively [6]. The ethnical background acts as an important factor for the D and I allele frequencies. The prevalence of the D allele is 39.1% in Asians, 56.2% in Caucasians, and 60.3% in individuals of African ancestry [7].
ACE measurement is widely used to support the diagnosis of sarcoidosis. Normal or decreased ACE activity does not rule out sarcoidosis, as only about 60-70% of patients exhibit elevated ACE activity. However, normal ACE activity can occur even in early, chronic, or inactive diseases when cells do not produce increased amounts of ACE [8]. Raised activities have been reported in a number of other conditions like Gaucher's disease, leprosy, tuberculosis and pathologic conditions involving lung and liver diseases. [5]

Method

Enzymatic photometric test that uses 4-hydroxyhippuryl-L-histidyl-L-leucine as substrate.

In a first step 4-hydroxyhippuryl-L-histidyl-L-leucine is cleaved by ACE into 4-hydroxyhippuric acid which is subsequently cleaved into 4-hydroxybenzoic acid and glycine by hippuricase. In-situ generated hydrogen peroxide is used in the presence of peroxidase (POD) to oxidize and condensate 4-hydroxybenzoic acid and 4-aminoantipyrine to produce a colored substance. The increase in absorbance is directly proportional to the ACE activity present in the sample [9].

4-Hydroxyhippuryl-L-histidyl-L-leucine —(ACE)→ 4-Hydroxyhippuric acid + L-Histidyl-L-leucine

4-Hydroxyhippuric acid —(Hippuricase)→ 4-Hydroxybenzoic acid + Glycine

4-Hydroxybenzoic acid + H_2O_2 + 4-Aminoantipyrine —(POD)→ Quinoneimine dye

One unit of ACE activity is the amount of enzyme that catalyzes 1.0 µmol of 4-hydroxyhippuric acid from the substrate 4-hydroxyhippuryl-L-histidyl-L-leucine per minute under the enzyme-specific conditions.

Reagents

Components and Concentrations

R1:	4-Hydroxyhippuryl-His-Leu	> 2 mmol/L
R2:	Buffer	> 50 mmol/L
	Peroxidase	> 15 kU/L
	Aminoantipyrine (4-AA)	> 10 mmol/L
	Hippuricase	> 20 kU/L

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored unopened at 2 – 8°C and contamination is avoided. Do not freeze.

After initial opening, the reagents should be placed on the analyzer and used until onboard stability is reached. Remaining reagents must be discarded after the onboard stability has been exceeded.

Warnings and Precautions

1. ⚠ For professional use only.
2. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. The reagents contain material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
4. ACE-inhibitors for treatment of high blood pressure can lead to falsely low results in patient samples [10,11].
5. In very rare cases, samples of patients with gammopathy might give falsified results [12].
6. Icteric, hemolyzed or lipemic samples may interfere (Note: Details in section Performance Characteristics).
7. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
8. Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.
9. The summary of safety and performance (SSP) may be accessed on the website of the European Database on Medical Devices (EUDAMED) via the following link: https://ec.europa.eu/tools/eudamed
10. Please refer to the safety data sheets (SDS) and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
11. The reagent is validated by the manufacturer on BioMajesty® JCA-BM6010/C. Use on other systems requires validation by the user.

Waste Management

Refer to local legal requirements for chemical disposal regulations as stated in the relevant SDS to determine the safe disposal.

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Reagent Preparation

The reagents are ready to use.

A maximum lot-to-lot variation of ± 25% has been defined.

Materials Required

General laboratory equipment

Commercially available NaCl 0.9% (w/v)-solution, with pH ranging from 4.5-7.0.

Specimen

Human serum or lithium heparin plasma

Do not use EDTA as an anticoagulant as it inhibits the ACE activity [13].

Only use suitable tubes or collection containers for specimen collection and preparation.

When using primary tubes, follow the manufacturer's instructions.

Stability in serum/plasma [14]:

1 day	at	20 – 25°C
7 days	at	4 – 8°C
1 year	at	-20°C

Only freeze once. Discard contaminated specimens.

Caution/Note: When interpreting the results, it must be taken into account that ACE inhibitors significantly lower ACE-activity depending on the dose administered.

Assay Procedure

Basic settings for BioMajesty® JCA-BM6010/C

Wavelength	505/805 nm
Temperature	37°C
Measurement	Endpoint
Sample/Calibrator	6.0 µL
Reagent 1	80 µL
Reagent 2	20 µL
Addition reagent 2	Cycle 19 (286 s)
Absorbance	Cycle 24/38 (354 s/546 s)
Calibration	Linear

Calculation

With Calibrator

$$\text{ACE [U/L]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Cal.}} \times \text{Activity Cal. [U/L]}$$

Conversion Factor

ACE [U/L] x 0.0167 = ACE [µkat/L]

Calibrators and Controls

DiaSys TruCal ACE is recommended for calibration. Calibrator values have been made traceable to the molar extinction coefficient of the FAPGG method from Beneteau B, Baudin B et al. [15]. Lot specific uncertainties can be obtained on request. Use DiaSys TruLab ACE Level 1 and Level 2 for internal quality control. All target values of the controls are traceable to DiaSys reagent/calibrator system. Quality control must be performed after calibration. Control intervals and limits have to be adapted to the individual requirements of each laboratory. Results must be within the defined ranges. Follow the relevant legal requirements and guidelines. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.		Kit size	
TruCal ACE	1 7600 99 10 046	3	x	1 mL
TruLab ACE Level 1	5 9300 99 10 046	3	x	1 mL
TruLab ACE Level 2	5 9310 99 10 046	3	x	1 mL

Performance Characteristics

Data evaluated on BioMajesty® JCA-BM6010/C

Measuring range from 5 U/L up to 175 U/L. Linearity ≤ 5 U/L is given within ± 30%, linearity > 5 U/L within ± 10%. When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.	
Limit of detection**	5 U/L
Limit of quantitation**	5 U/L
Onboard stability	12 weeks
Calibration stability	9 days

Interference by	**Interferences ≤ 15% up to**	**Analyte concentration [U/L]**
Ascorbic acid	50 mg/dL	38.0
	50 mg/dL	93.9
Bilirubin (conjugated)	30 mg/dL	32.4
	30 mg/dL	85.1
Bilirubin (unconjugated)	12.5 mg/dL	37.1
	12.5 mg/dL	97.7
Hemoglobin	200 mg/dL	32.3
	200 mg/dL	86.1
Hemolysis	600 mg/dL	33.0
	600 mg/dL	87.2
L-Cysteine	35 mg/dL	34.9
	35 mg/dL	88.1
Lipemia (triglycerides)	1000 mg/dL	33.2
	1000 mg/dL	91.2
Magnesium	30 mg/dL	35.3
	30 mg/dL	96.0
N-acetylcysteine (NAC)	500 mg/L	35.1
	500 mg/L	95.5
Uric acid	23.5 mg/dL	35.3
	23.5 mg/dL	88.5
Zinc	200 µg/dL	35.1
	200 µg/dL	96.2

Precision			
Repeatability (n=80)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [U/L]	29.1	64.6	121
CV [%]	1.60	1.38	1.60
Within-laboratory (n=80)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [U/L]	29.1	64.6	121
CV [%]	3.58	2.87	2.77
Reproducibility (n=75, no. of instruments=3)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [U/L]	38.0	55.2	145
CV [%]	5.30	4.67	2.80

Method comparison (n=100)	
Test x	Competitor ACE (ARCHITECT)
Test y	DiaSys ACE FS (BioMajesty® JCA-BM6010/C)
Slope	0.845
Intercept	8.84 U/L
Coefficient of correlation	0.970

Trueness
Since neither a reference material nor a reference method is available for the measurement of ACE activity, the trueness is demonstrated by a method comparison with an established method on the market (refer to the section Method comparison).

** according to CLSI document EP17-A2, Vol. 32, No. 8

Reference Range

In a healthy population, ACE values are usually expected to be < 14.7 IU/L (95th percentile) [16].

In this study, the cut-off value of 14.7 IU/L was defined for optimum sensitivity (78.1%) and specificity (81.7%) for detecting sarcoidosis. With a 14% sarcoidosis prevalence in the genotype DD, this results in a PPV of 23% and an NPV of 98.2% [16].

Positive likelihood LR+ = (Sensitivity/(1-Specificity)) ≥ 4.27

Negative likelihood LR- = ((1-Sensitivity)/Specificity) ≤ 0.27

NOTE: Serum ACE activity is strongly dependent on the genotype of the patient.

A study of 150 apparently healthy individuals (19 - 66 years) addressed this topic and investigated reference intervals for ACE activity depending on the genotype. The general reference range was defined at 13.3 - 63.9 U/L and the corresponding intervals for different genotypes were 12.3 – 65.6 U/L (DD), 9.5 – 49.5 U/L (ID) and 9.6 – 28.7 U/L (II) [6]. Due to the relationship between ACE genotype frequencies in different populations and geographic regions, the reference intervals given are for orientation purposes only.

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

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