

Angiotensin Converting Enzyme

FAP Method

Quantitative determination of Angiotensin Converting Enzyme (ACE) in serum or plasma IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD

Early methods for measuring ACE activity used the natural substrate Angiotensin I and products of the reaction were detected by bioassay, radioimmunoassay, HPLC or chemical methods. The use of hippuryl-L-histidyl-L-leucine as a substrate led to the development of more manageable spectrophotometric and spectrofluorimetric assays for ACE, however, these methods were still not ideally suited to automated analysis. The ACE reagent is based on the FAP method. In this method the direct substrate N-[3-(2-furyl)acryloyl]-L-phenylalanyl-glycylglycine (FAPGG) is hydrolysed to FAP and Glycylglycine. The hydrolysis of FAPGG by ACE results in a decrease in absorbance at 340nm.



CLINICAL SIGNIFICANCE

ACE is a halide activated membrane bound exopeptidase that has a central role in the control of blood pressure. ACE catalyses the conversion of Angiotensin I to the powerful vasoconstrictor Angiotensin II and also inactivates circulating Bradykinin. ACE is present in the vascular beds of most organs, however, the highest levels are found in the endothelial cells of pulmonary capillaries. Lung ACE is considered to be the principal source of the serum enzyme. The presence (I) or absence (D) of a 287 base pair fragment on the gene for ACE gives rise to three ACE genotypes, II, DD and ID. Since the discovery of the I/D polymorphism, further studies have shown that serum ACE activity is influenced by genotype. DD individuals have nearly twice the ACE activity of II individuals, with values from ID individuals being intermediate.

The measurement of serum ACE is widely used to aid in the differential diagnosis of clinically active pulmonary Sarcoidosis and for monitoring the effectiveness of steroid therapy. ACE measurement is also becoming widely used for monitoring the effects of ACE inhibitors in the treatment of hypertension and heart failure.

REAGENTS

R	Goods buffer, pH 8.2	80 mmol/L
	FAPGG	0,50 mmol/L
	Sodium azida	< 0,1 %

PREPARATION

The reagent is ready to use.

CALIBRATION

It is recommended to use the ACE Calibrator.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C.

After opening, the reagent is stable for 30 days when properly capped immediately after each opening and stored at 2-8°C protected from light.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 37° C (± 0.1°C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum: use non haemolysed serum.

Plasma: as ACE is inhibited by EDTA, heparinised plasma is a suitable specimen.

Stability: samples may be stored for 7 days at 2-8°C or 1 year at -20°C.

PROCEDURE

- Assay conditions:
Wavelength: 340nm
Cuvette: 1 cm light path
Constant temperature: 37°C
- Adjust the spectrophotometer to zero with distilled water or air.

- Pipette into a cuvette:

	Sample	Calibrator
R (µL)	1000	1000
Sample (µL)	100	
Calibrator		100

- Mix and incubate 5 minutes at 37°C.
- Read initial absorbance of the sample and calibrator (A₁), start the stopwatch and read absorbance exactly after 5 minutes (A₂).
- Calculate the difference of absorbance for the sample and calibrator (A₁ - A₂).

CALCULATIONS

$$\frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Calibrator}}} \times \text{Conc. Calibrator} = \text{U/L of ACE}$$

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: ACE Control

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES¹

At 37°C: 13,3 – 63,9 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: 5,8 - 120 U/L. If the sample concentration exceeds this value, dilute the sample 1:10 with NaCl 9 g/L and multiply the result by 10.

Sensitivity: The minimum detectable concentration of ACE with an acceptable level of precision was determined as 2,4 U/L.

Precision:

	Intra-assay (n=60)			Inter-assay (n=84)		
	Mean (U/L)	36,6	63,2	110,2	40,4	64,6
SD	0,7	0,9	0,9	1,2	1,8	1,9
CV (%)	2,0	1,4	0,8	2,9	2,7	1,7

Accuracy: Results obtained using Audit Diagnostics reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results were the following:

Correlation coefficient (r)²: 0,976.

Regression equation: y = 0,98x - 0,56.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Haemoglobin up to 100 mg/dL, free bilirubin up to 13 mg/dL, conjugated bilirubin up to 26 mg/dL and lipaemia, measured as triglycerides, up to 400 mg/dL, do not interfere.

ACE inhibitors, such as Captopril and Teprotides will inhibit serum ACE activity.

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

BIBLIOGRAPHY

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