

ACE (Angiotensin converting enzyme)

UV determination of Angiotensin Converting enzyme in serum or plasma

TEST SUMMARY

The angiotensin converting enzyme (ACE) catalyzes the hydrolysis of the substratum N-[3-(furyl)acryloyl]-L-Phenylalanyglycylglycine to N-[3-(furyl)acryloyl]-L-Phenylalanyne and glycylglycine.

The hydrolysis is associated to an absorption reduction which is been valued at 340 nm and is proportional to the enzymatic activity.

SAMPLES

Serum or plasma heparinate.
Stability: 4 days at 2- 8°C, 6 months at -20°C.

REAGENTS

Reagent 1: N-[3-(furyl)acryloyl]-L-Phenylalanyglycylglycine 28 mmol/l

Reagent 2: Buffer pH 8,4; Preservatives and stabilizers.

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatisation. Automatic Micropipette.

Cuvette in optical glass or monouse in optical polystyrene. Centrifuge. Water bath.

PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

REAGENTS PREPARATION

Reagents are stable at 2-8°C until expiration on label.

Reconstitute the contents of a vial of Reagent 1 with 4,2 ml of Reagent 2. Shake carefully until complete solubilisation.

Stability: 20 days at 2-8°C away from light source.

PROCEDURE

Method	fixed time
Wavelength	340 nm
Lightpath:	1 cm
Reading	against distilled water
Temperature	37°C
Reaction time	5 minutes
Sample/Reagent	1/10

WARNING: The reading with spectrophotometer is been led in a zone of the substratum spectrum in which even a modest change in wavelength determines a considerable change of the extinction coefficients.

For a well use of the kit, is necessary check accurately the wavelength calibration and the instrument sensibility.

For this aim the use of Control ACE is necessary.

Work Reagent	1 ml
Sample	0.1 ml

Mix and incubate at 37°C.

After 5 minutes read A1 absorbance and after exactly 5 minutes from first reading read A2 absorbance.

CALCULATION

ACE Activity (U/l) $(\Delta A \text{ 5 min.}) \times 2588$

Other commercially kits for the determination of ACE, using the same method, using a different factor calculation.

In order to compare the results obtained from the LTA kit with those obtained from other kits, you must multiply by 0.61.

EXPECTED VALUES

35 - 114 U/l

Every laboratory should establish own reference intervals in accordance with own population.

CALIBRATION/ QUALITY CONTROL

It is suggested to perform an internal quality control. For that aim on request, are available the following control sera:

CC03930 2 x 1 ml
Control-Set ACE Normal/Pathological

CC03940 2 x 1 ml
ACE Calibrator

NOTES

- ACE is a metalloprotein and so is essential avoid the employ of chelators in sample preparation.
- The reaction volumes could be changed respecting the proportions.
- In the activity calculation is been employed the following relation:

$$U/L = (A1 - A2) \times [(Vt \times 100) / (\Delta \epsilon \times d \times Vc \times t)]$$

where:

Vt : total volume;

$\Delta \epsilon$: change of extinction coefficient at 340 nm;

d : layer solution thickness ;

Vc : sample volume;

t : incubation time in minutes.

The formula for instance conditions quoted up becomes:

$$U/L = (A1 - A2) \times [(1,1 \times 1000) / (0,85 \times 1 \times 0,1 \times 5)] = (A1 - A2) \times 2588$$

The $\Delta \epsilon$ is being determined using spectrophotometers from research. Is possible that with clinical analysers the $\Delta \epsilon$ assumes a different value, with consequent change of the U/L values in normal and pathological subjects.

The calculation of $\Delta \epsilon$ for the employed instrument is possible using Control-Set ACE.

- It's opportune that each laboratory determines it's own normal values.
- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

TEST PERFORMANCE

Precision

Intra-assay (n = 10)	Mean (U/l)	SD (U/l)	CV%
Sample 1	44.26	1.1017	2.49
Sample 2	94.31	2.2673	2.40
Sample 3	178.69	4.6903	2.58

Inter-assay (n = 10)	Mean (U/l)	SD (U/l)	CV%
Sample 1	71.83	3.0811	4.29
Sample 2	144.48	4.3083	2.98

Sensitivity/cut-off

Cut-off as from 7 U/l.

Methods comparison

A comparison with a commercial available product gave the following results in a comparison on 103 samples:

ACE LTA = y
ACE competitor = x
n = 103

$$y = 1,27142 + 1,03417 \quad r = 0,99739$$

Linearity

The method is linear up to 250 U/l.

For activities superior to 250 U/l dilute one sample volume with a physiological solution volume, repeat the determination and multiply the result by 2.

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC03900

Reagent 1 6 vials (liofphile)
Reagent 2 2 x 15 ml









REFERENCES

Harjanne A. Clin. chem. 30 (1984) 901
Neels h.M..Clin. chem. 30 (1984) 901

MANUFACTURER

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SYMBOLS

	Only for IVD use
	Lot of manufacturing
	Code number
	Storage temperature interval
	Expiration date
	Warning, read enclosed documents
	Read the directions
	Biological risk

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