

ANGIOTENSIN CONVERTING ENZYME (ACE)

REF 305-10

10 x 10 ml

For other languages
 Pour d'autres langues
 Für andere Sprachen
 Para otras lenguas
 Per le altre lingue

Para outras línguas
 Για τις άλλεςλώσσες
 For andre sprog


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INTENDED USE

ACE Reagent is for use in the quantitative kinetic determination of angiotensin converting enzyme (ACE) activity in serum or plasma at 340 nm.

SUMMARY

Angiotensin converting enzyme (ACE, EC3.4.15.1, dipeptidyl carboxypeptidase) is a glycoprotein peptidyl dipeptide hydrolase that cleaves histidylleucine dipeptide from angiotensin I, a relatively inactive decapeptide. The latter is converted to the potent vasoconstrictor, angiotensin II. ACE also inactivates bradykinin. Elevated levels of ACE activity occur in serum of patients with active sarcoidosis,¹ and occasionally in premature infants with respiratory distress syndrome, in adults with tuberculosis, Gaucher's disease, leprosy, and in many other pathologic conditions involving lung and liver diseases.²

Significantly low levels were reported by Siefkin et al.² in many acute and chronic cases of lung injuries. Serial measurements of ACE in 71 patients showed that significantly decreasing levels over successive days were associated with a very high mortality rate. A single ACE measurement does not necessarily predict the presence or extent of lung injury, or aid in diagnosis of prognosis. However, serial levels are of value prognostically.

Several methods have been devised for measuring ACE activity including radioimmunoassay³ and competitive enzyme-linked immunoassay.⁴ The procedure described herein is a rapid, convenient spectrophotometric method utilizing the synthetic tripeptide substrate N-[3-(2-furyl)acryloyl]-L-phenylalanyl-glycylglycine (FAPGG).⁵

PRINCIPLE

The following reaction is catalyzed by ACE:



FAPGG is hydrolyzed to furylacryloylphenylalanine (FAP) and glycylglycine. Hydrolysis of FAPGG results in a decrease in absorbance at 340 nm. The ACE activity in the sample is determined by comparing the sample reaction rate to that obtained with the ACE Calibrator.

REAGENT

ACE REAGENT, 10 x 10 ml, 305-10.

When lyophilized reagent is reconstituted according to instructions it will contain FAPGG, 0.5 mmol/L, with stabilizer and buffer, pH 8.2.

PRECAUTIONS:

ACE Reagent is for "in vitro diagnostic use". Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state and federal laws. For professional use only. In case of damage, do not use.

ACE Reagent is IRRITATING to eyes and respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

The following instruction should be adhered to when opening the red flip-seal cap as it has a sharp edge after opening:

- A tweezers, needle-nose pliers, forceps, de-cappers, spatula or similar type of object should be used to open and peel off the flip-seal from the vial. When doing this action, ensure it is done outwards, away from the body.
- Latex gloves should also be worn to provide further protection to the user.

PREPARATION:

Reconstitute ACE Reagent with volume of deionized water indicated on vial label. If the reagent is to be used in a discrete analyzer, please refer to the respective application procedure. After addition of water, stopper the vial and immediately mix several times by inversion.

STORAGE AND STABILITY:

Store dry reagent refrigerated (2-8°C). Reagent is stable until the expiration date shown on the label. Reconstituted reagent is stable for 8 hours at room temperature (18-26°C) and for at least 30 days refrigerated (2-8°C).

DETERIORATION:

Discard the vial if dry reagent exhibits caking due to possible moisture penetration, does not dissolve completely or if the solution appears turbid.

DISCRETE ANALYZER APPLICATIONS

Please contact Trinity Biotech Technical Services Department for more information regarding applications procedures for ACE.

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection be carried out in accordance with current CLSI document M29. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Serum or heparinized plasma is recommended for analysis⁶. Serum samples are stable for 7 days when stored refrigerated at 2-8°C and 4 months when stored frozen at -20°C⁷. Heparinized plasma samples are stable for 4 weeks when stored frozen at -20°C or -80°C^{14,15}.

INTERFERING SUBSTANCES:

ACE activity is inhibited by EDTA and by heavy metal ions that may serve to replace the zinc ion of the enzyme⁶. Gadolinium contrast reagents have been reported to inhibit ACE activity^{11,12,13}. Upon administration of the angiotensin converting enzyme-inhibitory drug, captopril, currently used for treating hypertension, ACE serum activity is markedly reduced but usually returns to normal levels in about 12 hours.⁹ Administration of other such drugs may produce a similar response.⁹

MANUAL PROCEDURE

MATERIALS PROVIDED:

ACE Reagent

MATERIALS REQUIRED BUT NOT PROVIDED:

- ACE Calibrator, Catalogue No. 305-50
- Spectrophotometer capable of accurately measuring absorbance at 340 nm
- Cuvettes with optical properties suitable for use at 340 nm
- Pipetting devices for the accurate delivery of volumes required for the assay
- Timer
- Constant temperature cuvette chamber

PROCEDURE:

The temperature of the reaction mixture should be maintained at 30°C or 37°C.

1. Prepare ACE Reagent.
2. Reconstitute ACE Calibrator according to the instructions in its package insert.
3. Pipette 1.0 ml ACE Reagent Solution into each of two cuvettes labeled TEST and CALIBRATOR and bring to reaction temperature.
4. Add 0.1 ml specimen to cuvette labeled TEST. Add 0.1 ml ACE Calibrator to cuvette labeled CALIBRATOR. Mix by inversion.
5. Place cuvettes in constant temperature cuvette compartment and wait approximately 5 minutes.
6. Read and record the absorbances (A) of TEST and CALIBRATOR at 340 nm vs water as reference. These are INITIAL A's.
7. Exactly five minutes later, again read and record the absorbances. These are FINAL A's.

NOTES:

1. To demonstrate that the reaction is linear, take an additional reading 2.5 minutes after the INITIAL A. The absorbance changes for the two 2.5-minute intervals should be approximately the same.
2. If a recording spectrophotometer is available, the time required for the test may be considerably reduced. The ΔA per 5 minutes for TEST and CALIBRATOR may be obtained directly from the linear portion of their respective curves.

CALIBRATION:

The procedure is linear up to an ACE activity of 120 U/L. A calibrator included with each series of assays must be used to calculate ACE activity of tests.

QUALITY CONTROL:

Two serum preparations are currently available for control purposes. These include ACE Control-N, Catalogue No. A6040, and ACE Control-E, Catalogue No. A7040, containing normal and elevated levels of ACE activity, respectively.

Quality Controls should fall within their assigned ranges, otherwise the test run is invalid.

All performance data is generated from studies using serum as the sample matrix.

CALCULATIONS

ΔA per 5 min (TEST) = INITIAL A TEST - FINAL A TEST

ΔA per 5 min (CALIBRATOR) = INITIAL A CALIBRATOR - FINAL A CALIBRATOR

$$\text{ACE (U/L)} = \frac{\Delta A/5 \text{ min TEST}}{\Delta A/5 \text{ min CALIBRATOR}} \times \text{Activity of Calibrator}$$

One unit of ACE activity is defined as that amount of enzyme that will catalyze formation of one micromole of FAP per minute under the conditions of assay.

EXAMPLE:

The following absorbance values were obtained at 37°C using an ACE Calibrator with an activity of 50 u/l at 37°C.

	TEST	CALIBRATOR
INITIAL A	1.370	1.370
FINAL A	1.340	1.346
ΔA per 5 min	0.030	0.024

$$\text{ACE (U/L)} = \frac{0.030}{0.024} \times 50 = 62 \text{ U/L}$$

EXPECTED VALUES

Fasting blood samples from 56 clinically healthy adult males and females were assayed for ACE activity by the described method. The mean value obtained for the 56 individuals was 30 U/L. Normal range was calculated as the mean \pm 2 SD (SD=11). There was no difference in values obtained for males and females. Expected values are reported as higher in children and young adults under the age of 19 years than in older adults.¹⁰

ACE (U/L)
8 - 52 (37°C)
5 - 33 (30°C)

Values obtained by this procedure cannot be compared directly with those obtained by other procedures employing different conditions. The expected values range is provided as a guide. It is strongly recommended that each laboratory establish an expected range characteristic for the local population.

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY STUDIES:

For Intra Reproducibility, ten replicate assays were run on three serum samples of varying ACE activity on each of three platform analysers. For Inter Reproducibility, three serum samples of varying ACE activity were run on ten separate occasions in duplicate on each of three platform analysers.

Sample	Intra Reproducibility								
	Platform Analyser 1			Platform Analyser 2			Platform Analyser 3		
	1	2	3	1	2	3	1	2	3
Mean ACE (U/L)	24	58	98	27	53	97	21	65	109
Standard Deviation	1.9	3.4	1.3	3.8	3.7	1.9	2.0	1.8	1.7
%CV	7.9	5.8	1.4	13.8	6.9	1.9	9.4	2.8	1.6
No. Of Assays	10	10	10	10	10	10	10	10	10
Sample	Inter Reproducibility								
	1	2	3	1	2	3	1	2	3
	1	2	3	1	2	3	1	2	3
Mean ACE (U/L)	47	71	140	56	93	133	35	43	143
Standard Deviation	3.7	5.0	6.5	2.9	4.4	4.1	3.1	3.2	7.4
%CV	7.8	7.0	4.7	5.1	4.7	3.0	8.8	7.3	5.2
No. Of Assays	20	20	20	20	20	20	20	20	20

CORRELATION STUDIES:

Fifty two sera with ACE values ranging from 8-251 U/L were assayed by the described procedure and by the method described by Harjanne.⁷ The resulting correlation coefficient was 0.981 and the regression equation had a slope of 1.2 and a y-intercept of 8.0 U/L. Mean values were 76 U/L by the method of Harjanne and 53 U/L by the described procedure.












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ORDERING INFORMATION

Catalogue No.	305-10	
ACE REAGENT, 305-10	10 x 10 ml	
REQUIRED REAGENT		
Catalogue No.	Item	Quantity
305-50	ACE CALIBRATOR	6 x 1 ml
OPTIONAL REAGENTS		
Catalogue No.	Item	Quantity
A6040	ACE CONTROL-N (Normal)	6 x 1 ml
A7040	ACE CONTROL-E (Elevated)	6 x 1 ml

GUIDE TO SYMBOLS

 Use-by date	 Temperature limit
 Batch Code	 Consult instructions for use
 Catalogue number	 Manufacturer
 <i>In vitro</i> diagnostic medical device	Recon. Reconstitute with
 Water	 ACE Reagent
 Reproductive toxicity	 www.trinitybiotech.com eIFU indicator



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