Infinity™ ACE Liquid Stable Reagent
(Angiotensin Converting Enzyme)

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Measuring Range	:	1 - 100 U/L
Specimen Type	:	Serum or Plasma
Method	:	Kinetic/Rate
Reagent Preparation	:	Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		
	Consult instructions for use		Manufactured by

INTENDED USE
This reagent is intended for the in vitro quantitative determination of Angiotensin Converting Enzyme (ACE, EC3.4.15.1, dipeptidyl carboxypeptidase I) in human serum or plasma.

CLINICAL SIGNIFICANCE[1,2,3,4,5]
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 polymorphisim, 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.

METHODOLOGY[1,2,5]
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 spectrophotoemtric and sepctroflouorimetric assays for ACE, however, these methods were still not ideally suited to automated analysis.
The Infinty ACE reagent is based on the method first described by Holmquist et al. In this method the direct substrate N-[3-(2-furyl)-acryloyl] – L-phenyl-alanylglyclglycine (FAPGG) is hydrolysed to FAP and Glycylglycine as follows:

$$\text{FAPGG} \xrightarrow{ACE} \text{FAP + Glycylglycine}$$

The hydrolysis of FAPPG by ACE results in a decrease in absorbance at 340nm.

REAGENT COMPOSITION

Active Ingredients	Concentration
FAPGG	> 0.40 mmol/L
Tris buffer	55 mmol/L

Also contains non-reactive fillers and stabilisers.

pH 8.2 ± 0.1 at 19 - 22°C

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt thoroughly wash the affected areas with water. Reagent contains sodium azide which may react with copper and lead plumbing. Flush with plenty of water when disposing. For further information consult the Infinity ACE Liquid Stable Reagent Material Safety Data Sheet.

REAGENT PREPARATION
The reagent is supplied ready to use.

STABILITY AND STORAGE
Prior to use:
When stored at 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

Once the Reagent is Opened:
When stored capped at 2-8°C, the reagent is stable until expiry.

Indications of Reagent Deterioration:
- Turbidity; and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING
Serum: Use non haemolysed serum.
Plasma: As ACE is inhibited by EDTA, heparinised plasma is a suitable specimen.
Storage: ACE is a relatively stable enzyme. Serum samples may be stored for 1 day at 20 - 25°C, 7 days at 4 - 8°C [9]. Plasma samples may be stored for at least one week refrigerated and several months frozen[5].

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyser specific consumables, eg: sample cups.
- Thermo Scientific ACE assayed control material (TR85101).
- Thermo Scientific ACE Calibrator (TR85201).

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	340 nm
Assay Type	Kinetic
Direction	Decrease
Sample : Reagent Ratio	1 : 10
eg: Sample Vol	30 µL
Reagent Vol	300 µL
Delay/Lag Time	300 Seconds
Read Time	300 Seconds
Reagent Blank Limits	Low 0.0 AU
(340nm, 1cm lightpath)	High 2.0 AU
Measuring Range	1 -100 U/L
Analytical Sensitivity (340nm, 1cm lightpath)	0.084 ΔmA/min per U/L

CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

$$\text{ACE} = \frac{\Delta\text{Abs/min of Unknown}}{\Delta\text{Abs/min of Calibrator}} \times \text{Calibrator Value}$$

Example:
ΔAbs/min of Unknown = 0.0015
ΔAbs/min of Calibrator = 0.0033
Value of Calibrator = 66 U/L

$$\text{ACE} = \frac{0.0015}{0.0033} \times 66 = 30 \text{ U/L}$$

NOTES
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.

CALIBRATION
Calibration is required. Thermo Scientific ACE calibrator (TR85201) is recommended. For calibration frequency on automated instruments refer to the instrument manufacturer's specifications. However, calibration stability is contingent upon optimum

Thermo SCIENTIFIC

instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert.
Recalibration is recommended at anytime if one of the following events occurs:-

- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and elevated control should be run as unknown samples:-

- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

1. Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 725 mg/dL.
   Free Bilirubin: No interference from free bilirubin up to 222 µmol/L (13 mg/dL).
   Conjugated Bilirubin: No interference from conjugated bilirubin up to 342 µmol/L (20 mg/dL).
   Lipaemia: No interference from lipaemia, measured as triglycerides, up to 11.3 mmol/L (1000 mg/dL).
2. For a more comprehensive review of factors affecting ACE assays refer to the publication by Young.[6]
3. ACE inhibitors, such as Captopril and Teprotides will inhibit serum ACE activity.[2,5]
4. ACE is inhibited by EDTA.[5]

EXPECTED VALUES

At 37°C 8 - 52 U/L

The quoted values should serve as a guide only. It is recommended that each laboratory verify this range or derive a reference interval for the population it serves.[7]

PERFORMANCE DATA

The following data was obtained using the Infinity ACE Liquid Stable Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated over a period of 20 days using two levels of commercial control and following the NCCLS EP5-T procedure.[8]

Within Run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	35	90
SD (U/L)	2.4	2.0
CV (%)	6.9	2.3

Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (U/L)	35	90
SD (U/L)	3.7	5.1
CV (%)	10.8	5.7

METHOD COMPARISON

Comparison studies were carried out using a similar commercially available reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	108
Range of sample results	1 - 114 U/L
Mean of reference method results	39.2 U/L
Mean of Infinity ACE results	34.3 U/L
Slope	0.961
Intercept	-3.3 U/L
Correlation coefficient	0.966

MEASURING RANGE

When run as recommended the assay is linear between 1 and 100 U/L of ACE.

ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of this assay is 0.084 ΔmA/min per U/L (1cm light path, 340 nm).

REFERENCES

1. Muller BR. Ann Clin Biochem 2002;39:436-43
2. Studdy PR and Bird R. Ann Clin Biochem 1989;26:13-18
3. Beneteau B et al. Clin Chem 1986;32:884-6
4. Butter J and Stuart S. CLin Chem 1993;39:312-6
5. Maguire GA and Price CP Ann Clin Biochem 1985;22:204-10
6. Young DS, Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990; 3-37
7. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.
8. National Committee for Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices. NCCLS, 1984, NCCLS Publication EP5-T.
9. Recommendations of the Working Group on Preanalytical Quality of the German Society for Clinical Chemistry and the German Society for Laboratory Medicine. The Quality of Diagnostic Samples. 1st Edition 2001:16.

Fisher Diagnostics
a division of Fisher Scientific Company, LLC
a part of Thermo Fisher Scientific Inc.
Middletown, VA 22645-1905 USA
Phone: 800-528-0494
540-869-3200
Fax: 540-869-8132

EC REP WMDE
Bergerweg 18
6085 AT Horn
The Netherlands

REF

Reorder Information

Catalogue No.		Configuration
TR85056		2 x 28 mL
TR85101	Control	6 x 1 mL
TR85201	Calibrator	6 x 1 mL

JL840733 (R0)