SYMBOL KEYS

2°C 8°C	Store at 2°C to 8°C		Manufacturer	IVD	*In vitro* Diagnostic Medical Device	R1	Enzyme Reagent
	Use by	LOT	Batch Number/ Lot Number		Consult Instructions for use	R2	Starter Reagent
	Date of Manufacture	REF	Catalogue Number		This side up	C	Calibrator

Manufactured by:

Coral Clinical Systems

A Division of Tulip Diagnostics (P) Ltd.

Plot No. M-46, Phase III B, Bldg. No. D, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

ADA2/0820/VER-01

ADAZYME™-LS

FOR THE DETERMINATION OF ADENOSINE DEAMINASE ACTIVITY IN SERUM, PLASMA AND BIOLOGICAL FLUIDS BY ENZYMATIC METHOD

SUMMARY

Adenosine deaminase (ADA) is an endogenous tissue enzyme which is released into the serum in patients with different types of malignancies and infections, including viral hepatitis, infectious mononucleosis and tuberculosis. In pleural fluid elevated ADA levels are very commonly associated with tuberculosis. In CSF, ADA levels are elevated in cases of tuberculous meningitis. Increased concentration of serum ADA has shown the potential of usable screening test and can be used in the diagnosis of liver diseases in combination with ALT or γ-GT (GGT) tests.

PRESENTATION

REF (REF)	1102310010	1102310025
Pack size	10 ml	25 ml
R1	8 ml	20 ml
R2	2 ml	5 ml
C	1 ml	1 ml
Pack insert	1	1

REAGENT

ADAZYME™-LS contains reagents for laboratory use only.
ADAZYME™-LS comprises of:

a) R1 - ADAZYME™-LS : Enzyme Reagent, ready to use.
b) R2 - ADAZYME™-LS : Starter Reagent, ready to use.
c) C - ADAZYME™-LS : Calibrator (Lyophilized).

PRINCIPLE

The ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine **by purine nucleoside phosphorylase** (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by **xanthine oxidase** (XOD). H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of **peroxidase** (POD) to generate a Quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction is as follows.

ADA –ENZYMATIC REACTION

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Inosine} + NH_3$$

$$\text{Inosine} + Pi \xrightarrow{\text{PNP}} \text{Hypoxanthine} + \text{Ribose-1-phosphate}$$

$$\text{Hypoxanthine} + H_2O + O_2 \xrightarrow{\text{XOD}} \text{Uric acid} + H_2O_2$$

$$H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{POD}} H_2O + \text{Quinone dye } (\lambda\text{max } 546\text{nm})$$

One unit of ADA is defined as the amount of ADA that generates one µmole of inosine from adenosine per min at 37ºC.

REFERENCE RANGE

The ADA activities in 60 healthy human serum samples were found to be in the range of 0-15 U/L. For Pleural fluid values were found to be in the range of 0-30 U/L, for C.S.F. values were found to be in the range of 0-9 U/L, for pericardial fluid values were found to be $\leq$ 40U/L and for Ascitic fluid $\leq$ 30U/L. It is recommended that each laboratory establish its own range of reference values.

STORAGE AND STABILITY

1. Store the ADAZYME™-LS kit at 2-8°C, away from light.
2. Stability of the ADAZYME™-LS kit is as per the expiry date mentioned on the label.

NOTE

1. It is important that kit components from the same lot are used for achieving accurate and reproducible results. Do not intermix reagents from different lots.
2. The sequence of addition of reagents should be followed meticulously for achieving accurate results.

ADDITIONAL MATERIAL REQUIRED

Test tubes, test tube stand, waterbath/incubator (37°C), distilled or deionised water, variable volume pipettes, spectrophotometer with filter at 540-550 nm at 37°C or colorimeter with yellow filter, stopwatch.

REAGENT PREPARATION

ADAZYME™-LS is an enzymatic assay system that can be used for both manual method and automated chemistry analyser. The Enzyme Reagent (R1) is in liquid ready to use form. The Calibrator (C) is in (lyophilized) form : Reconstitute with 1 ml of distilled water. Reconstituted Calibrator is stable for 15 days at 2-8 ºC.

SPECIMEN COLLECTION AND PREPARATION

Collect specimen prior to use of antimicrobial agent. Wherever possible, indicate clearly that patient is on antitubercular drugs. **CSF:** Collect as much as possible in a syringe, clean skin with alcohol before aspirating specimen. **Body fluids:** Disinfect the site and collect specimen with aseptic precautions. **Serum, Plasma**: No special preparation of the patient is required prior to sample collection by approved techniques. It is recommended to use fresh sample specimen for testing. Do not use haemolyzed, contaminated or turbid sample specimens.

ADA is reported to be stable in serum for 3 days at 2-8°C and in biological fluids for 2 days at 2-8°C, as after this, ammonia may be released in the samples even without any microbial contamination.

Assay Procedure

Wavelength : 546nm
Temperature : 37 ºC
Light path : 1 cm

For Manual Method

Pipette into clean dry test tube as follows:

Addition sequence	
R1	0.400 ml
Test Sample/ Calibrator	0.010 ml
Incubate at assay temperature (37 ºC) for 3 minutes and add	
R2	0.100 ml

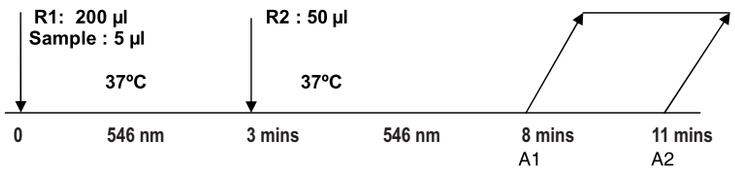
Mix well and incubate for 5 minutes at 37 ºC. Read the initial absorbance A1. Measure the change in absorbance per minute (ΔA/min) for the next 3 minutes.

Calculations

ADA Activity in U/L = $\frac{\Delta AT}{\Delta AC}$ X Concentration of Calibrator

ΔAT- mean absorbance/minute of the test sample
ΔAC- mean absorbance/minute of the Calibrator

For Automated System



For Automated Clinical Chemistry analyzer, Programming/ System Parameters has to be adjusted as per the instrument setup.

Linearity

The linearity of the procedure is from 0 - 200 U/L.

Limitations

Assay is specific for ADA and has no detectable reaction with other nucleosides. The reagent solution should be clear, and if turbidity is seen then the reagent may have deteriorated.

If the sample ADA activity is greater than 200U/L, the sample should be diluted with normal saline. The result should be multiplied by the dilution factor.

SAFETY PRECAUTIONS AND WARNINGS

1. Reagent R1 is light sensitive. Store in a dark place.
2. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those Biosafety in Microbiological and Biomedical Laboratories (HHS publication Number [CDC] 93-8395).
3. As with any diagnostics test procedures, results should be interpreted considering all other test results and clinical status of the patient.
4. Avoid ingestion and contact with skin and eyes.
5. Do not use the reagents after the expiration date mentioned on the label.

System Parameters :

Reaction Type (Mode)	Fixed time Kinetics
Wave Length	546 nm
Zero Setting	Distilled Water
Incubation Temperature	37 °C
Incubation time	--
Delay Time	300 Secs
Read Time	180 Secs
Number of Reads	3
Interval	60 Secs
Sample Volume	0.010 ml
Reagent Volume (R1 +R2)	0.500 ml
Standard / Calibrator	Value mentioned on vial label
Factor	--
Reaction Slope	Increasing
Linearity	200 U/L
Units	U/L

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1) Use of Adenosine Deaminase for the diagnosis of Tuberculosis: A Review; Lakkana Boonyagars,M.D ; Sasisopin Kiertiburanakul, M.D.,MHS. (2) Laboratorial validation of an automated assay for the determination of adenosine deaminase activity in pleural fluid and cerebrospinal fluid.; Feres MC, Martino MC, Maldijian S, Batists F, Gabriel Junior A, Tufik S. (3) Kobayashi, Ikeda T, Marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease. Am. J. Gastroenterol, 88; 266-271 (1983). (4) Test book of clinical chemistry, Ed. By N. W. Tietz, W.B. Saunders Co., Philadelphia (1999). (5) Martinek R.C.,Clinical Chemistry 9, 620 (1963). (6) Kalkan A., Bult V., Erel O., Avci S., and Bingol N.K. : Adenosine deaminase and guanosine deaminase activities in sera of patients with viral hepatitis. Mem Inst. Oswaldo Cruz 94 (3) 383-386 (1999). (7) Burgess LJ, Martiz FJ. Le Roux I, et al Use of Adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax 50: 672-674 (1995). (8) The usefulness of adenosine deaminase in the diagnosis of Tuberculous Pericarditis.; Felipe Fransisco TUON(1), Vivian Iidada SILVA (1), Gisele M. Duboc de ALMEIDA (2), Leila ANTONANGELO (2) & Yeh Li HO (1). (9) Data on file: Tulip Diagnostics (P) Ltd.