NEO CHEM

ADENOSINE DEAMINASE (ADA)

(Enzyme cycling method)

KIT NAME	KIT SIZE
NEO CHEM - ADA	1X15 ML

NEOGENIX
DIAGNOSTICS PVT LTD

INTRODUCTION

Increased ADA activity has been observed in Tuberculosis, the most specific test is the positive bacterial culture of a patient's sample. This is cumbersome and time consuming. X-rays, smears for AFB and Tuberculin tests though comparatively rapid are not conclusive. Adenosine Deaminase (ADA) is an enzyme widely distributed in mammalian tissues, particularly in T-Lymphocytes. Increased levels of ADA are found in various forms of tuberculosis making it a marker for the same. Though ADA is also increased in various infectious diseases like infectious mononucleosis, Typhoid, Viral Hepatitis, initial stages of HIV, and in case of malignant tumors, the same can be ruled out clinically.

METHOD PRINCIPLE

The Kit utilizes enzymatic and kinetic reactions to measure the ADA activity (U/L) in human serum or plasma.

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Inosine} + Nh3$$

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

$$\text{Hypoxanthine} + 2H_2O + 2O_2 \xrightarrow{\text{XOD}} \text{Uric acid} + H_2O_2$$

$$H_2O_2 + 4\text{-AA} + \text{EHSPT} \longrightarrow H_2O_2 + \text{Quinone dye}$$

Adenosine is converted to inosine then hypoxanthine by the series deamination with adenosine deaminase(ADA) and purine nucleoside phosphorylase (PNP). Hypoxanthine then reacts with water and oxygen and forms uric acid and hydrogen peroxide. In the end hydrogen peroxide is reduced to water and quinone dye is produced by reacting with 4-aminoantipyrine and N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT). The process is quantified by measuring the absorbance at 550 nm in a kinetic reaction. The rate of increase in absorbance at 550 nm is directly proportional to the ADA activity in the sample.

KIT CONTENTS

Reagent Name	Pack Size
R1 - ADA regent	1 X 10 ml
R2 - ADA reagent	1 x 5 ml
R3 - Calibrator	0.5 ml

Please refer the calibrator value mentioned in the vial.

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 2 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination.

WORKING REAGENT PREPARATION AND STABILITY

Assay can be performed with use of separate R1-ADA and R2-ADA reagents or with use of working reagent. For working reagent preparation mix gently 2 parts of R1-ADA with 1 part of R2-ADA. Avoid foaming.

Stability of working reagent : 2 weeks at 2-8°C
10 days at 15-25°C

CONCENTRATIONS IN THE TEST

Glycine buffer pH 7.2	80 mmol/L
Xanthine Oxidase	800 mmol/l
Nucleoside Phosphorylase	50 U/L
4-Aminiantipyridine	2.0 mmol/l
Adenosine	0.0 mmol/l
Peroxidase	600 U/L
EHSPT	2 mmol/L

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 546 nm
- Thermostat at 37°C
- General laboratory equipment

SPECIMEN

Serum, heparinized plasma may be assayed. Venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant.

PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analyzers. Programmer Sheets are available on request.

Wavelength	546 nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Calibrator (C)	Test (T)
R1 ADA reagent	720 µl	720 µl
R3 Calibrattor	20 µl	-
Sample	-	20 µl
R2 ADA reagent	360 µl	360 µl

Mix well measure the absorbance the increase in absorbance every 60 seconds interval for 3 readings and calculate the ΔA/min at 37°C

CALCULATION

ADA concentration U/L = ΔA(T) / ΔA(S) x Calibrator concentration

REFERENCE VALUES

For Serum, plasma, pleural, pericardial & ascitic fluids

Normal	upto 43 U/L
suspect for MTB	43 to 62 U/L
strong suspect for MTB	above 62 U/L

For CSF

Normal	Less than 11 U/L
suspect for MTB	11 to 12.35 U/L
strong suspect for MTB	above 12.35 U/L

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS

• **Linearity:** up to 200 U/L. Dilute the sample approximately and re-assay if ADA activity exceeds 200 U/L. Multiply result with dilution factor.

LITERATURE

1. Kobayashi F, Ikeda T. Marumo F, Sato C: Adenosine Deaminase isoenzymes in liver disease, Am, J.Gastroenterol.88:266-271 (1993)
2. 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)
3. Burgees L J, Matitz F.J,Le Roux I,etal. Use of Adenosine Deaminse as a diagnostic tool for tuberculosis pleurisy. Thorax50:672-674(1995)
4. Lakkana B., Sasisopin K: Use of adenosine deaminase for the diagnosis of tuberculosis: A review J. infect, Dis Antimicrob Agents 2010: 27:111-8
5. Delacour H., Sauvanet C., Ceppa F., Burnat P.: Analytical Performances of the Diazyme ADA assay on the Cobas 6000 system. Clinical Biochemistry 43 (2010 1468-1471).

WASTE MANAGEMENT

Please refer to local legal requirements.

SYSTEM PARAMETERS

Method	Kinetic
Wavelength	546 nm
Zero Setting	Distilled water
Temperature Setting	37°C
Incubation Temperature	37°C
Incubation Time	----
Delay time	300 secs
Read time	180 secsc
No. of Reading	3
Interval time	60 secs
Sample Volume	0.02 ml (20 µl)
Reagent Volume	1.08 ml (1080 µl)
Calibrator Concentration	Refer calibrator vial
Units	U/L
Factor	----
Reaction slope	Increasing
Linearity	200 U/L