ADA
Enzymatic Kinetic Method

INTENDED USE:
This reagent kit is intended for ***"in vitro"*** quantitative determination of Adenosine Deaminase (ADA) activity in serum and plasma samples and other body fluids.

CLINICAL SIGNIFICANCE:
Tuberculosis occurs worldwide. 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.

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,O,) by xanthine oxidase (XOD). H,O, is further reacted with N-Ethyl-N-(-2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AAP) in the presence of peroxidase (POD) to generate quinine dye which is monitored in a kinetic manner.

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

$$\text{Inosine} + Pi \longrightarrow \text{Hypoxanthine} + \text{Ribose-I-phosphate}$$

$$\text{Hypoxanthine} + 2\,H_2O + 2\,O_2 \xrightarrow{POD} \text{Uric Acid} + 2\,H_2O_2$$

$$2\,H_2O + \text{4-AAP} + \text{EHSPT} \longrightarrow 2\,H_2O + \text{Quinone dye}$$

REAGENT COMPOSITION:
Reagent 1 : Enzyme Reagent
Reagent2: Substrate Reagent

MATERIALS REQUIRED BUT NOT PROVIDED:
- Clean & Dry Glassware.
- Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

SAMPLES:
Serum or heprinised plasma may be assayed. Venous blood should be collected and handeled anaerobically. Do not use citrate or oxalate as anticoagulant.

WORKING REAGENT PREPARATION & STABILITY:
Reagents are stable until their expiration date when stored at 2 - 8 ^{0}C.

GENERALSYSTEMPARAMETERS:

Reaction type	Kinetic Reaction (Increasing)
Wavelength	546nm
Light Path	1Cm
Reaction Temperature	37°c
Blank/ Zero Setting	With Distilled Water
Reagent Volume	R1- 360µ1 R2-180µl
Sample Volume	10 µl
Lag/ Delay Time	300Sec.
Read Time	180Sec.
Interval Time	60Sec.
Factor	1725
Linearity	200 U/l

Assay Procedure: For Serum / plasma / pieural / CSF / pericardia! or, ascetic fiuids

Reagent R1	360 µl
Serum/plasma/pleural/ CSF,pericardial or ascitic fluid	10 µl

Mix and incubate for 5 min at 37°C.

Reagent R2	180 µl

Mix and after 300 second incubation, measure the increase in absorbance every minute during 3 minutes at 37°C.

Determine the t.A/min.

CALCULATION: At 546 nm with 1cm Light path

ADA Activity in Serum / plasma / pleural / pericardia! / CSF

or ascitic fiuid (U/l) = *Ll* A/min. x 1725

LINEARITY:
Reagent is Linear up to 200 U/1.
Dilute the sample appropriately and re-assay if ADA Activity exceeds 200 U/l . Multiply result with dilution factor.

REFERENCE NORMAL VALUE:
For Serum, plasma, pieural, pericardial & ascetic fluids

Normal	upto 43 U/l
Suspect for MTB	43 U/l to 62 U/l
Strong Suspect for MTB	Greater than 62 U/l

ForCSF

Normal	Less Than 11 U/l
Suspect for TBM	11 U/l to 12.35 U/l
Strong SuspectforTBM (Tuberculous Meningitis)	Greater than 12.35 U/l

The reference values are only indicative in nature. Every laboratory should establish its own normal ranges.

QUALITY CONTROL:
For accuracy it is necessary to run known controls with every assay.

BIBLIOGRAPHY:

1. Kobayashi F, Ikeda T, Marumo F, Sato C : Adenosine deaminase isoenzymes in liver desease. Am. J. Gastroenterol. 88: 266 -271(1993)
2. Kalkan A., Bull V., Ere! O.,Avci S., and Bingo! N.K.: Adeonsine deaminase and guanosine deaminase activities in scra of patients with viral hepatitis. Mcm Inst. Oswaldo Cruz 94 (3) 383·386 (1999)
3. Burgess L J,Matitz F J, Le Roux I, et al. Use ofadenosine deaminase as a diagnostic tool for tuberculosis pleurisy. Thorax 50: 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., Sauvanct C., Ceppa F., Burnat P.: Analytical Performances of the Diazyme ADA assay on the Cobas 6000 system. Clinical Biochemistry 43 (2010 1468-1471)

Gene+

GENIX BIOTECH

Gala No 217, 218, 219 Building No.3,
2nd Floor Jay Vijay Industrial Estate
Naigaon East, Mumbai.