ADENOSINE DEAMINASE

(PNP-XOD/KINETIC Method)

Intended Use

Adenosine Deaminase is a reagent kit used for the determination of ADA activity in serum, plasma or cerebrospinal fluid (CSF) based on PNP-XOD/Kinetic method. The reagents are for *in-vitro* diagnostic use.

Principle

The Kit utilizesenzymatic and kinetic reactions to measure the ADA activity (U/L(in human serum or plasma

$$\text{Adenosine} + H_2O \xrightarrow{ADA} \text{Inosine} + Nh_3$$

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

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

$$H_2O_2 + \text{4-AA} + \text{EHSPT} \longrightarrow 2H_2O + \text{Quinone dye}$$

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 546 nm in a kinetic reaction. The rate of increase in absorbance at 546 nm is directly proportional to the ADA activity in the sample.

Reagent provided

1. R1 – ADA Reagent
2. R2 – ADA Reagent
3. R3 - Calibrator

Reagent storage and stability

ADA reagent and calibrator are stable till the expiry date stated on the container label.

Specimen collection and preservation

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

Assay guidelines for Analyzers

Reaction type	Kinetic
Reaction slope	Increasing
Flow cell Temperature	37°C
Wavelength	546 nm
Zero setting with	Distilled water
Delay Time	300 sec.
Interval Time	60 sec.
No. of Readings	4
Sample volume	10 µl (0.01 ml)
Reagent volume 1	360 µl
Reagent volume 2	180 µl
Calibrator Concentration	Refer on Calibrator vial
Low Normal	22
High Normal	40
Linearity	200 U/L

Assay guidelines for Manual procedure

Reagents	Standard	Sample
ADA Reagent 1	360 µl (0.36 ml)	360 µl (0.36 ml)
Calibrator	10 µl (0.01 ml)	-
Sample	-	10 µl (0.01ml)
Mix and Incubate for 5 minutes at 37°C		
ADA Reagent 2	180 µl (0.18 ml)	180 µl (0.18 ml)

Mix & after the initial delay of 300 sec., record the absorbance of the test at an interval 60 sec. for the next 180 sec. at 546 nm . determine the mean change in absorbance per minute and calculate the test result.

Calculation

$$\text{ADA Con. (U/L)} = \frac{\Delta A\ (T)}{\Delta A\ (S)} \times \text{Concentration Of Calibrator}$$

Normal Range

	NORMAL	SUSPECT	STRONG SUSPECT
Serum/Plasma	< 22 U/L	22 - 40 U/L	> 40 U/L
Body Fluids (Pleural/Pericardial/Ascitic)	< 30 U/L	30-40 U/L	> 40 U/L
CSF	< 9 U/L	9 - 12 U/L	> 12 U/L

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

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.

References

1. Kobayashi F, Ikeda T-MarumoF, Sato C:Adenosine Deaminase ISO enzymes in liver disease, Am,J. Gastroenterol.88:266-271 (1993)
2. 2. Kalkan A,. bultV, 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 AntimicrobAgents 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)

Symbols

IVD In Vitro Diagnostics. Caution. Keep away from sun light. Date of Manufacture.

LOT Batch No. Read Instructions. Fragile. Product Expiry Date.

CONT Content. Storage Temperature. Keep Dry. Manufactured By.

REF Catalogue No.

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