ADENOSINE DEAMINASE (Enzymatic-Kinetic-Method)

Invitro Diagnostic reagent kit for quantitative determination of ADA activity in human serum/plasma/CSF sample on Photometric System.

Reagent

Reagent 1: Enzyme Solution
Reagent 2: Substrate Solution

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 TOOS and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction scheme is shown below.

$$\text{Adenosine} + H2O \xrightarrow{\text{ADA}} \text{Inosine} + NH3$$

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

$$\text{Hypoxanthine} + 2H_2O + 2O \xrightarrow{\text{XOD}} \text{Uric Acid} + 2H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{TOOS} \xrightarrow{\text{POD}} 4H_2O + \text{Quinone dye}$$

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

Summary

ADA is an enzyme catalyzing the de-amination reaction from adenosine to inosine. The enzyme is widely distributed in human tissues, especially high in T-lymphocytes. Elevated serum ADA activity has been observed in patients with acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, liver cirrhosis, viral hepatitis and hepatoma. Increased ADA activity was also observed in patients with tuberculous effusions. Determination of ADA activity in patient serum may add unique values to the diagnosis of liver diseases in combination with ALT or γ-GT (GGT) tests. ADA assay may also be useful in the diagnostics of tuberculous pleuritis.

Storage Instructions and Reagent Stability

Reagent is stable up to the end of the indicated month of expiry, if stored at 2°– 8°C, protected from light and contamination is avoided. Do not freeze the reagents!

Components and concentrations

Reagent: Tris-HCl (pH: 8.0); 4-AA: 2mM; PNP: 0.1 U/L; XO: 0.2 U/L; POD: 0.6 U/L; Adenosine: 10mM; preservative.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The Reagents Enzyme Solution And Substrate Solution.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Cerebrospinal fluid or Serum, heparin plasma or EDTA plasma separate at the latest 1h after blood collection from cellular contents.

7 days at 2° –8°C
30 days at –20°C
Only freeze once! Discard contaminated specimens.

Assay Procedure

Wavelength 546 nm (540 – 550 nm)
Optical path 10 mm
Temperature 37°C

	For Sample
Reagent 1	360 µL
Sample	10 µL
Mix and incubate at 37°C for 5 minutes, then add Reagent 2	
Reagent 2	180 µL
Mix and Incubate for 5 minutes and read absorbance (A1) and again after 3 minutes (A2).	

Calculations

ΔA = A2 – A1

U/L of ADA in the sample = Sample ΔA/min x Factor(1743)

Quality Controls

For internal quality control any normal and abnormal controls should be assayed with each batch of samples.
Each laboratory should establish corrective action in case of deviations in control recovery.

Warnings and Precautions

1. Cuvette and other glassware must be cleaned thoroughly after being used for other assays. In case of automated measurement refer to the instrument manual for special washing programs.
2. In very rare cases, samples of patient's with gammopathy might give falsified results.
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.

Performance Characteristics

Measuring Range

The test has been developed determine ADA within a measuring range from 1 – 200 U/L. If such value is exceeded the sample should be diluted 1+1 with NaCl solution (9 g/L) and results multiplied by 2.

Linearity/limit of Maximum Detection

The higher limit of detection is 200 U/L.

Sensitivity/Limit of Detection

The lower limit of detection is 1 U/L.

Interferences

No interference was observed by, Ascorbic acid up to 30 mg/dL, Bilirubin up to 40 mg/dL and Triglycerides up to 1000 mg/dL.

Precision

Intra-assay n = 20	Sample 1	Sample 2	Sample 3
Mean[U/L]	11.37	30.25	42.95
SD[U/L]	0.19	0.43	0.41
CV [%]	1.70	1.42	0.95

Inter-assay n = 20	Sample 1	Sample 2	Sample 3
Mean[U/L]	11.15	30.70	42.10
SD[U/L]	0.13	0.27	0.41
CV [%]	1.15	0.88	0.97

Method Comparison

A comparison of Precision Biomed ADA (y) with a commercially available test (x) using 15 samples gave following results:
$y = 1.025x - 1.019$; $R^2 = 0.996$

Reference Range

For Serum, plasma, pieural, pericardial & ascetic fluids

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

For CSF

Normal	Less Than 11 U/l
Suspect for TBM	11 U/l to12.35 U/l Strong
Suspectfor Meningitis)	TBM Greaterthan12.35U/l (Tuberculous

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

Quick Reference

Parameter	Adenosine Deaminase(MTB)
Reaction Type	Increasing
Mode	Kinetic
Wavelength	546 nm
Path length	10 mm
Temperature	37°C.
Reagent 1	360 µL
Sample	10 µL
1st Incubation time	300 sec.
Reagent 2	180 µL
Delay	300sec.
Rate	180 sec.
Normal range	Serum/Plasma pieural, pericardial & ascetic fluids 0 – 43 U/L
	CSF – 0 – 11 U/L
Linearity	200 U/L
Sensitivity	1 U/L

Pack Size

Cat No.	Configuration	Pack
ADA00015	Reagent R1 - 1 x 10mL Reagent R2 - 1 x 5mL	15mL
ADA00030	Reagent R1 - 2 x 10mL Reagent R2 - 2 x 5mL	30mL

Literature

1. Kobayashi F, Ikeda T, Marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease. Am. J. Gastroenterol. 88: 266-271 (1993).
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3. Burgess LJ, Maritz FJ, Le Roux I, et al. Use of adenosine deaminase as a diagnositic tool for tuberculous pleurisy. Thorax 50: 672-674 (1995).
4. 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.
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6. Porcel, JM.: Handling Pleural Fluid Samples for Routine Analyses. Derleme. June 2013; 19-22.
7. Al-Shammary FJ.: Adenosine Deaminase Activity in Serum and Pleural Effusions of Tuberculous and Non-Tuberculous Patients. Biochemistry and Molecular Biology International 43(4) 763-779 (1997)
8. Bielsa S., Esquerda A., Palma RM, et al.: Influence of Storage Time on Pleural Fluid Adenosine Deaminase Activity. Clin. Lab. 2014; 60: 501-504.
9. Gupta BK, Parul G, Haren B, et al.: Cerebrospinal fluid Adenosine deaminase: its evaluation as a marker for diagnosing tu-berculous meningitis in paediatric patients. IOSR-JDMS Jan.-Feb. 2013; 4(1): 21-24.

Version : ADA/00

IVD In Vitro Diagnostic Use | See Pack Insert For Procedure | Single Use only | CE
Temperature Limit | Manufacturer's Address | Manufacturing Date | Expiry Date | LOT Lot Number

Manufactured in India by :
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