

# High-Q Adenosine Deaminase (ADA)

PNP-XOD/Kinetic Method

## Intended Use:

Adenosine deaminase (ADA) assay kit is for determination of ADA activity in human Serum, Plasma samples, Pleural, Pericardial, Peritoneal and Cerebro Spinal Fluids.

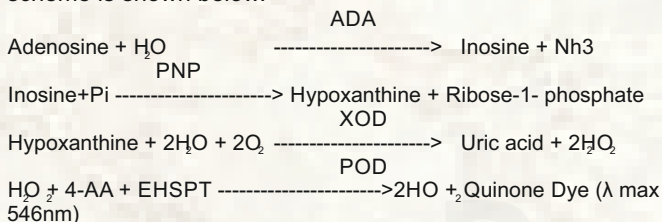
## Clinical Significance

ADA is an enzyme catalyzing the deamination 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  $\gamma$ -GT (GGT) tests. ADA assay may also be useful in the diagnostics of tuberculous pleuritis.

## Assay Principle:

The ADA assay consists of four steps:

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<sub>2</sub>O<sub>2</sub>) by xanthine oxidase (XOD). H<sub>2</sub>O<sub>2</sub> 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 quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction scheme is shown below.



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

## Reagent Composition:

### Active Ingredients

#### Reagent 1

	Concentration
Tris HCl, pH 8.0	50 mMol/L
4-AA	4 mMol/L
PNP	100 U/L
XOD	700 U/L
Peroxidase	2000 U/L
Surfactant	0.1%

#### Reagent 2

Tris-HCl, pH 7.0	50 mMol/L
Adenosine	10 mMol/L
EHSPT	2 mMol/L

ADA Control (Available Optionally) Adenosine deaminase (bovine liver) and BSA

## Reagent Preparation:

Liquid two-reagent system, ready-to-use for both manual method and automated chemistry analyzers (kinetics).

ADA Calibrator is in the liquid format and is ready to use. Calibrator is stable till the expiry date mentioned on labels when stored properly at 2-8°C

ADA Controls are available as Liquid Stable and Ready to use format and are stable till the expiry date mentioned on the labels

## Reagent Stability and Storage:

Reagents are stable until their expiration date when stored at 2-8°C.

## Specimen Collection and Handling

Serum or Heparinized Plasma may be assayed. Ideally, venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant. Plasma and serum, after prompt separation from cells or clot, should be kept tightly stoppered. ADA content of blood is stable for 1 week when stored at 2-4°C.

When the other body fluids (Pleural Fluid, Pericardial Fluid, Peritoneal Fluid, Cerebrospinal Fluid) are tested for ADA, ideal collection procedures should be followed.

## Calibration:

Calibrator with a known value printed on the labels can be used to calibrate and validate the ADA assay.

## Assay Procedure for the Fully Automated Analyzer

**System Parameters:** (Serum, Plasma, Pleural Fluid, Pericardial and Ascitic Fluid and CSF)

Reaction Type (Mode)	:	Fixed Time
Reaction Direction	:	Increasing
Wave Length	:	546 nm
Flow Cell Temp.	:	37°C
Zero Setting with	:	Distilled Water
Delay time	:	30 seconds
Measuring time	:	120 Sec
Reagent Volume (R1+R2)	:	360 $\mu$ l + 180 $\mu$ l
Sample Volume	:	10 $\mu$ l
Calibrator Concentration	:	On the label
Linearity	:	250
Units	:	U/L

Reagent	Calibrator	Sample/Control
ADA R1	360 $\mu$ l	360 $\mu$ l
Calibrator	10 $\mu$ l	----
Sample	—	10 $\mu$ l
<b>Mix and incubate for 5 Minutes at 37 °C</b>		
ADA R2	180 $\mu$ l	180 $\mu$ l

Mix well and read absorbances of Calibrator and Sample against distilled water at 546 nm (530-550 nm) as follows:

Initial absorbance A1 - exactly after 30 sec.

Final absorbance A2 - exactly 120 sec. after A1

Determine  $\Delta A$  for Calibrator (C) and Test (T)

## Calculations :

$$\text{ADA Conc.: (U/L)} = \frac{(A2-A1) \text{ Sample}}{(A2-A1) \text{ Calibrator}} \times \text{Calibrator Concentration}$$

## Quality Control:

Pariksha recommends that each laboratory should use ADA controls to validate the performance of ADA reagents. ADA controls are available from Pariksha Biotech Pvt Ltd.

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## Reference Range:

We have tested ADA activity in 400 Indian human samples (Sera and Body Fluids) and the following reference ranges for MTB were drawn out of ADA assay.

Serum, Plasma, Pleural, Pericardial & Ascitic Fluids	Normal	Less than 43 U/L
	Suspect for MTB	43 U/L to 62 U/L
	Strong Suspect for MTB	Greater than 62 U/L
C.S.F	Normal	Less than 11.00 U/L.
	Suspect for TBM	11 U/L to 12.35 U/L
	Strong Suspect for TBM (Tuberculous Meningitis)	Greater than 12.35 U/L

It is recommended that each laboratory should establish its own range of reference values.

## Result Interpretation:

Since High-Q Adenosine Deaminase is intended for the determination of Adenosine Deaminase in various disease conditions like Tuberculosis and Hepatic Disorders one has to clinically evaluate the disease condition before arriving at the diagnosis.

**Note:** Elevated levels of ADA have been reported in peritoneal, meningeal, pleural, pericardial effusions in several non tubercular diseases like Hepatic Cirrhosis, Typhoid fever, Infectious mononucleosis, Brucellosis and Bronchogenic carcinoma involving stimulation of cell mediated immunity. It is for the pathologist to clinically correlate and corroborate the results with the other diagnostic findings  
The above reference ranges can not be compared with Colorimetric Methods (Giusti Methods) of ADA Estimation where Ammonia is measured in the final reaction.

## Linearity:

The method is linear up to 250 U/L.  
Samples above this concentration should be diluted 1+1 with 0.9% NaCl solution and the result multiplied by 2.

## Interference:

Assay is not affected by serum bilirubin up to 31 mg/dl, hemoglobin up to 220 mg/dl, triglycerides up to 1000 mg/dl and ascorbic acid up to 4 mg/dl.

Analytical sensitivity (Lower detection limit) : 4 U/L

## Precision:

### Within-Run

n= 20	Level 1	Level 2
Mean (U/L)	31.53	35.45
SD (U/L)	0.17	0.17
CV (%)	0.54	0.48

### Between-Run

n= 20	Level 1	Level 2
Mean (U/L)	32.20	35.59
SD (U/L)	0.95	0.67
CV (%)	2.95	1.90

## Precautions:

1. Reagent R1 is light-sensitive. Store in a dark place.
2. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
3. Avoid ingestion and contact with skin and eyes.
4. Do not use the reagents after the expiration date labeled on the outer box.

## References:

1. Kobayashi F, Ikeda T, Marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease. Am. J. Gastroenterol. 88: 266-271(1993)
2. Burgess LJ, Maritz FJ, Le Roux I, et al. Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax 50:672-374(1995).
3. Kallkan 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).
















## Ordering Information

Ref./Cat. No.	Pack Size	Presentation
P- ADA-75	75 ml	50 ml R1 + 25 ml R2 with Calibrator

## Product Features:

- Liquid Stable, ready to use two reagents with calibrator.
- Kinetic reaction time 150 sec (30 Sec Delay+ 120 Sec Measuring).
- Linearity: 250 IU/L.
- Measuring Wavelength 546nm.
- Assay is not affected by serum bilirubin up to 31 mg/dl, Hemoglobin up to 220 mg/dl, triglycerides up to 1000 mg/dl and ascorbic acid up to 4 mg/dl.
- Pleural Fluid, Ascitic Fluid (Peritoneal Fluid), Pericardial Fluid, Cerebrospinal Fluid.
- Body Fluid, Serum or Heparinized Plasma are to be used as specimens based on the clinical condition.
- Available as multipurpose reagents and dedicated system packs.

## Symbols used with IVD devices

	Date of manufacture		Manufactured by
	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)		Reagent
	Calibrator Material		Batch code
	Temperature limitation (store at)		Control
	Consult instructions for use		Keep dry Keep away from rain
	Catalog Number		

eIFU Indicator



Pariksha's world inside  
SCAN TO EXPLORE MORE

Manufactured in India by :  
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