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PARAMCARE™

Adenosine Deaminase (ADA) Test Kit

PNP-XOD Method

Quantitative determination of ADA activity in human Serum / Plasma / other body fluids. Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Pack Size
ADA 25	1 X 25 ml
ADA 50	1 X 50 ml
ADA 100	1X100 ml
ADA 5000	1X5000 ml
ADA 10000	1X10000 ml

CLINICAL SIGNIFICANCE

ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. The enzyme is widely distributed in human tissues, and is 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 diagnosis of tuberculous pleuritis.

METHOD

Photometric Enzymatic Test.

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 (H2O2) by xanthine oxidase (XOD). H2O2 is further reacted ← with ← N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3- methylaniline(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.

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

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

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

$$H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{POD}} 2H_2O + \text{Quinone Dye}\ (\lambda\ \text{max}\ \ 546\text{nm})$$

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

REAGENT

Reagent 1 : Enzyme solution
Reagent 2 : Substrate solution
ADA Calibrator : Concentration is stated on Vial label

REAGENT PREPARATION

The reagents are provided in a ready to use format.

REAGENT STORAGE AND STABILITY

Reagents are stable until the expiry date shown on the label when stored tightly closed at 2 – 8 °C and if contaminations are prevented during their use

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

Serum or heparin plasma or other body fluids (Pleural Fluid, Peritoneal fluid, Pericardial fluid and CSF)

Stability:
7 days at 4 – 8°C
1 month at –20°C
Discard contaminated specimens. Only freeze once!

ASSAY PROCEDURE

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, standard and samples to room temperature 18 – 28 ºC, prior to analysis.

Automated Parameters	
Wavelength	546 nm
Measurement	Against DI Water
Reaction Temperature	37°C
Reaction Type	Fix time Kinetic
Reaction Direction	Increasing
Incubation	3 Min.
Sample Volume	10 µl
Reagent I Volume	400 µl
Reagent II Volume	200 µl
Delay/Lag/Time	300 Sec.(5min.)
Measuring Time	180 Sec.(3min.)
Linearity	200
Units	IU/L

MANUAL ASSAY PROCEDURE

Pipette into Test Tubes

REAGENT I	400 µl
SAMPLE	10 µl
Mix well and incubate for 3 mins at 37°C & Immediately Add	
REAGENT II	200 µl
Mix & aspirate. After the initial delay time of 300 seconds, record the abs., of the test at the interval of 60 seconds for the next 180 seconds at 546 nm. Determine the mean change in absorbance per minute and calculate the test result.	

SAMPLE DILUTIONS

- The method is linear to a concentration of 200 IU/L.
- If the concentration exceeds this value, the sample should be diluted 1:1 with 0.9% saline solution and reassayed. Multiply the result by 2.

CALCULATION

$$\text{ADA activity (IU/L)} = \frac{\Delta\text{O.D/min. of sample}}{\Delta\text{O.D/min. of Calibrator}} \times \text{Calibrator Conc.}$$

CALIBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable multi-calibrator is recommended.
It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
Control 1	30.79	0.78	2.52%
Control 2	144.27	0.76	0.53%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Control 1	30.81	0.36	1.18%
Control 2	143.11	1.85	1.29%

LINEARITY

The method is linear to a concentration of 200 IU/L.
If the concentration exceeds this value, the sample should be diluted 1:1 with 0.9% saline solution and reassayed. Multiply the result by 2.

Limit of detection: The limit of detection for Adenosine Deaminase is 4IU/L.

METHOD COMPARISON

A comparison of Adenosine Deaminase with a commercially available assay (x) using 58 samples gave following results: $R^2 = 0.9800$

REFERENCE VALUES

for serum, plasma, Pleural paracardial and Ascitic Fluid

Normal	up to 43 U/L
Suspect for MTB	43 to 62 U/L
Strong Suspect for MTB	greater than 62 U/L

For CSF

Normal	up to 11 U/L
Suspect for TBM	11 to 12.35 U/L
Strong Suspect for TBM	greater than 12.35 U/L

MTB (Mycobacterium Tuberculosis)
TBM (Tuberculous Meningitis)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

LIMITATION OF THE PROCEDURE

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

INTERFERENCE

- Ascorbic Acid: No interference found upto 50mg/dl.
- Hemoglobin: No interference found upto Hemoglobin 800mg/dl.
- Lipemia: No interference found upto 1000mg/dl.
- These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used.

BIBLIOGRAPHY

1. Kobayashi F, Ikeda T, Marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease.Am.J.Gastroenterol. 88:266-271(1993)
2. 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).

GLOSSARY OF SYMBOL

	Consult Instruction for Use
	Catalog Number
	Store between
	Manufacturer
	Keep away from sunlight

Paramcare Life Sciences Private Limited, G/F-12/13, Evershine-2,
Survey No. 307/3/1, Balitha N.H No 48, Vapi, Valsad, Gujarat, 396191.
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