TRUEchemie Adenosine Deaminase (ADA) Test Kit (PNP-XOD)

for the direct quantitative determination of Adenosine Deaminase (ADA) in human serum or plasma or CSF.

INTENDED USE

The TRUEchemie Adenosine Deaminase (ADA) Test Kit (PNP-XOD) is intended for "in vitro" quantitative determination of Adenosine Deaminase (ADA) activity in serum and plasma samples and other body fluids.

INTRODUCTION

Tuberculosis occurs world wide 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 increase in various infectious diseases like infectious mononucleosis, Typhoid, Viral Hepatitis, initial stages of HIV, and in case of malignant tumors, the same can be rule 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 (H2O2) by xanthine oxidase (XOD). H2O2 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 quinone dye which is monitored in a kinetic manner.

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

$$\text{Inosine} + \text{PI} \xrightarrow{\text{PNP}} \text{Hypoxanthine} + \text{Ribose-l-phosphate}$$

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

$$2H_2O_2 + \text{4-AAP} + \text{EHSPT} \xrightarrow{\text{POD}} 2H_2O + \text{Quinone dye}$$

PACK SIZE

Kit Size	**1 x 15 ml**
Cat. No.	**ADX391**
Kit contents	
1) ADA Reagent (R1)	1 x 10 ml
2) ADA Reagent (R2)	1 x 5 ml

REAGENTS COMPOSITION

1) ADA Reagent (R1):

Tris-HCl pH 8.0	: 25 mmol/L
4-AA	: 4 mmol/L
PNP	: 0.3 U/mL
XOD	: 0.4 U/mL
Peroxidase	: 0.1 U/mL

2) ADA Reagent (R2):

Tris -HCl pH 4.0	: 25 mmol/L
Adenosine	: 11mmol/L
EHSPT	: 4 mmol/L

REAGENT PREPARATION

Ready to use reagents.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.
2. Specimens should be considered infectious and handled appropriately.
3. Avoid ingestion. DO NOT PIPETTE BY MOUTH.
4. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate
5. The disposal of the residues has to be done as per local legal regulations.

REAGENT STORAGE & STABILITY

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2-8°C. Do not use reagents over the expiration date.

SPECIMEN COLLECTION AND STORAGE

Serum or heparinized plasma may be assayed. Venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant. Sample is stable for one week at 4°C

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes to accurately measure required volumes.
2. Test tubes/rack
3. Timer
4. 37°C heating block or water bath
5. Photometer capable of accurately measuring absorbance at 546 nm

TEST PROCEDURE

Wavelength	:	546 nm
Temperature	:	37°C

Prewarm the Reagent to reaction temperature.

Assay Procedure for serum/ plasma/pleural/ pericardial or ascitic fluids

	Blank (µl)	Sample (µl)
Distilled Water	500	-
ADA Reagent (R1)	-	360
Sample	-	10
Mix and incubate for 3 mins at 37°C		
ADA Reagent (R2)	-	180

Assay Procedure for CSF

	Blank (µl)	Sample (µl)
Distilled Water	500	-
ADA Reagent (R1)	-	360
Sample	-	40
Mix and incubate for 3 mins at 37°C		
ADA Reagent (R2)	-	180

Reading & Calculations

Blank the Photometer with D.I Water.
Mix and read the absorbance after 300 sec incubation. Measure the absorbance after every minute for 3 mins
ADA (U/L) = (ΔA/min.) x factor

Factor for CSF = 297
Factor for Serum/ plasma/pleural/pericardial or ascitic fluids = 1743

QUALITY CONTROL

Quality Controls are recommended to monitor the performance of automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

EXPECTED VALUE

Serum, Plasma, Pleural, Pericardial & Ascitic fluids	Normal	Less than 43 U/L
	Suspected For MTB	43 U/L to 62 U/L
	Strong Suspected for MTB	Greater than 62 U/L
CSF	Normal	Less than 11 U/L
	Suspected For MTB	11 U/L to 12.35 U/L
	Strong Suspected for MTB	Greater than 12.35 U/L

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

PERFORMANCE CHARACTERISTICS

Sensitivity: 0.02 U/L
Linearity: Up to 200 U/L under the described assay conditions. If the concentration is greater than linearity (200 U/L), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor. The linearity limit depends on the sample / reagent ratio, as well as the analyzers used.

PRECISION:

Intra-assay precision within run (n=10)	**Mean (U/L)**	**SD (U/L)**	**CV (%)**
Control Level - 1	30.7	0.2	0.7
Control Level - 2	62.1	0.6	1.0

Inter-assay precision run to run (n=12)	**Mean (U/L)**	**SD (U/L)**	**CV (%)**
Control Level - 1	30.0	0.3	1.1
Control Level - 2	56.5	0.4	0.7

The reagent was tested for 12 days, using two different ADA concentrations. The coefficient of variation was <5%.

AUTOMATED PROCEDURE

Appropriate program sheet is available for different analyzers upon request.

METHOD COMPARISON

Results obtained using TRUEchemie ADA reagent (y) did not show systematic differences when compared with another commercial reagent (x) with similar characteristics. The results obtained is below: The correlation coefficient (r^2) was 0.994 and the regression equation is y=0.982x+0.510. The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemoglobin (up to 800 mg/dL) do not interfere.
Intralipid (up to 1000 mg/dL) do not interfere.
Ascorbic acid (up to 50 mg/dL) do not interfere.

WASTE MANAGEMENT

Please refer to local regulation requirements.

SYSTEMS PARAMETERS

Mode	:	Kinetic
Factor	:	Serum-1743 / CSF-297
Wave length	:	546 nm
Units	:	U/L
Flow cell Temp	:	37°C
Blank	:	Distilled Water
Reagent volume (R1 & R2)	:	360 µL (R 1) + 180 µL (R2)
Sample volume	:	Serum : 10 µL / CSF: 40 µL
Lag time	:	300 sec. (5min.)
Read time	:	180 sec. (3 min.)
Normal serum	:	Less than 43 U/L
Normal CSF	:	Less than 11 U/L
Sensitivity	:	0.02
Linearity	:	200
Reaction Slope	:	Serum-Increasing / CSF-Decreasing

REFERENCES

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, Bult V, Erl O, Avei S. and Bingol N.K. Adeonsine deaminase and guanosine deaminase activities in sera of patients with viral hepatitis. Mem Inst. Oswaldo Cruz 94 (3)383-386 (1999)
3. Burgess L . J. Matitz F.J, Le Roux I, et al. Use of adenosine deaminase as a diagnostics tool for tuberculosis pleurisy. Thorax 50: 672-674 (1995)
4. Lakkana B, Sasisopin K: use of adenosine deaminase for the diagnostis of tuberculosis: A review J. infect Dis Antimicrob Agents 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)
6. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

Index of Symbols

Consult instructions for use		REF Catalogue number		Caution	
IVD *In vitro* diagnostic medical device		LOT Batch code		Non-sterile	
2°C 8°C Temperature limit 2-8 °C		Do not re-use		Use-by date	
Manufacturer		Date of manufacture		Keep dry	

Do not use if package is damaged — Keep away from sunlight

Athenese-Dx Pvt. Ltd.
Module No. 407 & 408, 4th Floor,
TICEL Bio Park II, No. 5, CSIR Road.
Taramani, Chennai-600113, India
Tel: +91-44-22541131
E-mail: info@athenesedx.com
Website: www.athenesedx.com

PI-ADX39 Rev. D
Effective date: 07.02.2024
English version