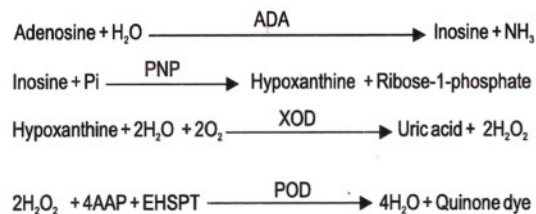


Introduction

1. AutoPure ADA is a reagent kit for direct quantitative determination of Adenosine deaminase (ADA) activity in human serum, plasma and other body fluids.
2. AutoPure ADA is a ready-to-use, two liquid reagent system.
3. With AutoPure ADA, the assay is linear upto 200 U/L.

Principle

ADA catalyses deamination of adenosine to inosine which is then 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 (4AAP) in the presence of peroxidase (POD) to generate quinone dye.



The intensity of the colour developed is directly proportional to the activity of ADA in the specimen and is measured kinetically.

Reagent Storage, Stability & Handling

AutoPure ADA is a ready-to-use, two liquid reagent system.

Shelf life

Stable till the expiry date indicated on the label, when stored at 2° - 8° C.

On - Board Reagent Stability

R1 : 28 days at 2° - 8° C.

R2 : 28 days at 2° - 8° C.

Protect the reagent from light and contamination.

Do not freeze the reagent.

Components & Concentration of Working Solution

Component	Concentration
R1	
• Tris	> 45 mM
• PNP	> 0.08 U/ml
• XOD	> 0.15 U/ml

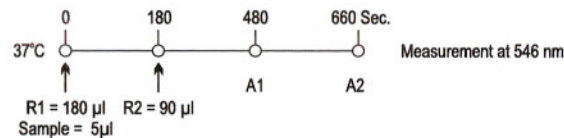
- Peroxidase > 0.40 U/ml
- Stabilizers, excipients & surface active agents
- R2
- Tris > 40 mM
- Adenosine > 8 mM
- EHSPT > 1 mM

Specimen Collection & Preservation

Collect sample using standard sampling tube. Serum or heparinized plasma can be used. Do not use citrate or oxalate as an anticoagulant. ADA in serum / plasma is stable for 1 week at 2° - 8° C. Centrifuge samples containing precipitate before performing assay.

Procedure

AutoPure ADA can be used on various automated analyzers. General procedure is as described below.



PROCEDURE FOR SEMI-AUTOMATED ANALYZERS

- Reaction Type Kinetic
- Reaction direction Increasing
- Wavelength 546 nm.
- Flowcell temperature 37°C
- Zero setting with Distilled water
- Delay time 300 seconds
- No. of readings 4
- Interval 60 seconds
- Sample volume 10 µl
- Reagent 1 (R1) volume 360 µl
- Reagent 2 (R2) volume 180 µl
- Factor 1743
- Linearity 200 U/L

Manual assay procedure :

Perform the assay as given below:

R1 0.360 ml (360 µl)
 Sample 0.010 ml (10 µl)

Mix and incubate for 3 minutes at 37°C.

R2 0.180 ml (180 µl)

Mix and aspirate. After the initial delay of 300 seconds, record the absorbance of the test at an interval of 60 seconds for the next 180 seconds at 546 nm. Determine the mean change in absorbance per minute and calculate the test results.

Calculation :
 Activity of ADA in U/L = ΔAbs./min. X 1743

Calculations

Fully automated system automatically calculates the ADA activity of each sample.

Application Sheet

For system applications, contact our local Accurex representative.

Calibration

For calibration, it is recommended to use ADA calibrator from Accurex. Other commercially available ADA calibrators have not been tested with this assay and may not be supported by AutoPure ADA. In case calibrator is not available, fix factor (1743) can be used.

Calibration frequency

Re - calibration is recommended

- Whenever the reagent lot is changed.
- As per the requirement of quality control procedures.

Quality Control

Each batch of AutoPure ADA is assayed with multiple quality control sera prior to release.

To ensure adequate quality control, it is recommended that the laboratory should use a normal and abnormal commercial reference control serum. It should be realized that the use of quality control material checks both the reagent and instrument functions together.

If the control values fall outside the specified limits, each of the below criteria should be cross - checked and corrected:

- Proper instrument function – wavelength setting, light source and temperature control.
- Cleanliness of probes and cuvettes.
- Bacterial contamination of wash water used by the instrument.
- Expiry date of the reagent kit.

Expected Values

Serum: 0 – 15 U/L
 Pleural fluid: 0 – 30 U/L
 CSF: 0 – 9 U/L

Note:

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own normal range. For diagnostic purposes, the ADA results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Performance Characteristics

Linearity

With AutoPure ADA, the assay is linear up to 200 U/L. Determine samples with higher concentrations via the rerun function. On instruments without rerun function, manually dilute samples with higher concentrations using 0.9% NaCl or distilled/deionized water. Multiply the result by the appropriate dilution factor.



Interference

There is no significant interference from samples containing up to 200 mg/dl of haemoglobin, 30 mg/dl of Bilirubin, 750 mg/dl of triglycerides and 4 mg/dl of ascorbic acid.

Precision

Reproducibility was determined using two levels of sera as shown below:

n=30

Serum pool	Within run			Between run		
	Mean U/L	SD U/L	%CV	Mean U/L	SD U/L	%CV
Low (11 U/L)	11.11	0.16	1.47	10.63	0.47	4.90
High (30 U/L)	30.74	0.45	1.45	29.62	0.59	2.00

References

1. Kobayashi F, Ikeda T, Marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease. *Am. J. Gastroenterol.* 88: 266 - 271 (1993).
2. Kallikan 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).
3. Burgess LJ, Maritz FJ, Le Roux I, et al. Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. *Thorax* 50: 672 - 674 (1995).
4. Lakkana B., Sasisopin K : Use of Adenosine deaminase for the Diagnosis of Tuberculosis. A review *J. infect. Dis Antimicrob Agents* 2010; 27:111-8.

AR.No.:1107

ASAD1-2014-08-001

ISO 13485, ISO 9001 CERTIFIED COMPANY

Accurex Biomedical Pvt. Ltd.

Head Office - Mumbai. Tel.: 91 (022) 67446744; Fax: 91 (022) 67446755
E-mail: accurex@vsnl.com; Website: www.accurex.org
Plant : G-54, MIDC Tarapur, Boisar, Thane - 401 506. INDIA.
Call us toll free on : 1800 209 8456