LiquiMAX Adenosine Deaminase (ADA)

PNP-XOD/Kinetic Method

ORDERING INFORMATION

Ref. No.	Pack Size	Presentation
AVADA-12	12 ml	R1 1 x 8 ml, R2 1 x 4 ml
AVADA-24	24 ml	R1 2 x 8 ml, R2 2 x 4 ml
AVADA-48	48 ml	R1 4 x 8 ml, R2 4 x 4 ml

INTENDED USE :

LiquiMAX (ADA) is an in-vitro diagnostic kit for the quantitative determination of ADA activity in human Serum, Plasma, Pleural, Pericardial, Peritoneal and CSF. This kit is a automated.

INTENDED USER:

Laboratory Technician

PRODUCT FEATURES

- Liquid stable, ready to use two reagents(with factor).
- Kinetic Factor = 1746
- Kinetic reaction time 150 sec (30 Sec Delay+ 120 Sec Measuring).
- Linearity: 250 U/L.
- Measuring Wavelength 546nm.
- Pleural Fluid,Ascitic Fluid (Peritoneal Fluid),Pericardial Fluid, Cerebrospinal Fluid.
- Fluid, Serum or Heparinized Plasma are to be used as specimens based on the clinical condition.

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 γ-GT (GGT) tests. ADA assay may also be useful in the diagnostics of tuberculous pleuritis.

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_2O) by xanthine oxidase (XOD). H_2O is 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.

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

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

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

$$H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{POD}} 2HO_2 + \text{Quinone Dye } (\lambda \text{ max } 546\text{nm})$$

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

STORAGE AND STABILITY

All the reagents are stable up to the expiry date mentioned on the labels when the proper storage conditions are maintained.

KIT COMPONENTS

1. ADA Reagent R1
2. ADA Reagent R2

COMPOSITION:

Active Ingredients	Concentration
Reagent 1	
Tris HCl, pH 8.0	55 mMol/L
4-AA	5 mMol/L
PNP	200 U/L
XOD	500 U/L
Peroxidase	4000 U/L
Surfactant	1%
Reagent R2	
Tris-HCl, pH 7.0	25 mMol/L
Adenosine	12 mMol/L
EHSPT	2.3 mMol/L

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

1. When the reagent is stored properly at 2-8ºC & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard any turbid reagent or blank reagent absorbance exceeds 0.3 at 546 nm against distilled water.

WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

SPECIMEN & COLLECTION STORAGE

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– 4ºC.

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

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

Reaction Type (Mode)	:	Kinetic
Reaction Direction	:	Increasing
Wave Length	:	546 nm
Flow Cell Temp.	:	37ºC
Zero Setting with	:	Distilled Water
Delay time	:	30 Seconds
Measuring time	:	120 Sec
Reagent Volume (R1+R2)	:	360 µl+ 180 µl
Sample Volume	:	10 µl
Factor	:	1746
Linearity	:	250
Units	:	U/L

TEST PROCEDURE:

Reageant R1	360µl
Sample (Serum, Plasma, Pleural Fluid Ascitic Fluid, CSF)	10 µl
Mix well and incubate for 5 minutes at 37°C	
Reagent R2	180µl

Immediately aspirate into the analyzer. Measure the change of optical density during the next 150 seconds (**Delay 30 seconds and measuring 120 seconds**) against distilled water at 546 nm.

CALCULATION

From the absorbance reading calculate delta Abs./min. and multiply by the corresponding factor

ADA activity(IU/ml) = delta Abs./min X 1746(Factor) (Serum, Plasma, Pleural fluid, Pericardial fluid, Ascitic fluid and CSF)

EXPECTED VALUES

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

Serum, Plasma, Pleural, Pericardial and Ascitic Fluid	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
CSF	Normal	Less than 11.0 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 Liquimax 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.

QUALITY CONTROL & CALIBRATION:

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

PERFORMANCE CHARACTERISTICS

1. Linearity

Linearity : 250 U/L.
Samples above this concentration should be diluted 1+1 with 0.9% NaCl solution and the result multiplied by 2.

2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 4 U/L

3. Interferences

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.

4. Precision:

Intra-Assay

(N=20)	Mean U/L	SD U/L	CV%
Control serum 1	31.56	0.17	0.54
Control serum 2	146.45	0.17	0.48

Inter-Assay

(N=20)	Mean U/L	SD U/L	CV%
Control serum 1	32.0	0.95	2.95
Control serum 2	147.59	0.67	1.90

5. Method Comparison:

A comparison of the LiquiMAX ADA (y) with a commercial obtainable assay (x) gave the following result : $y = 1.113x - 0.278$; $r = 0.990$

LIMITATIONS

Measuring range: 4-250 U/L. Determine samples having higher concentrations manually dilute with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

NOTES

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

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 patientswith vral hepatits. Mem Inst. Oswaldo Cruz 94(3) 383- 386 (1999).

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

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Manufactured in India by :
AVECON Healthcare Pvt. Ltd.
Transforming Research into Innovations
Plot No.: 338, Sector-2, Industrial Growth Centre, Saha, Ambala, Haryana (INDIA)-133104
E-mail : helpdesk@aveconhealthcare.com, Website : www.aveconhealthcare.com
Customer Care No. : +91 93065 12576, CIN No.: U24230HR2006PTC118875

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