LIQUIZYME

ADENOSINE DEAMINASE

(PNP-XOD Method)

BEACON

Code	Product Name	Pack Size
LS005A	Liquizyme Adenosine Deaminase	30 ml
LS005C	Liquizyme Adenosine Deaminase	90 ml

Intended Use

Diagnostic reagent for quantitative *in vitro* determination of Adenosine Deaminase in human serum.

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 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_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with 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.

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

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

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

$$2H_2O_2 + \text{4-AA} + \text{TOOS} \xrightarrow{\text{POD}} 2H_2O_2 + \text{Quinone dye (max 556 nm)}$$

Reagents

Reagent 1 : ADA R1 Reagent

Buffer	:	>80 mmol/L
4-AA	:	<2 mmol/L
PNP	:	<3 KU/L
Peroxidase	:	>0.6 KU/L

Reagent 2 : ADA R2 Reagent

Buffer	:	>50 mmol/L
Adenosine	:	<10 mmol/L
TOOS	:	<2 mmol/L

Reagent 3: ADA Calibrator

Refer vial label for concentration

Reagent Preparation

Reagents are liquid, ready to use.

Stability And Storage

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.

Precautions

Solution R1 and CAL contain Sodium Azide. 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 medical attention. All specimens used in this test should be considered potentially infectious.

Calibration

Recommend that this assay should be calibrated using the ADA Calibrator. Reagents and calibrator are ready to use.

Materials Required But Not Provided

- Clean & Dry container.
- Laboratory Glass Pipettes or Micro pipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

Storage And Stability

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

Reagent Deterioration:

Presence of particles and turbidity.

Quality Control

It's recommended to run normal and abnormal control sera to validate reagent performance.

Specimen And Stability

Fresh serum and non-hemolyzed serum or plasma, Pleural, Pericardial, Ascitic fluid and CSF can be used. Ideally, venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant.

Stability:

7 days : at 2-4°C

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Assay Procedure

1. Assay conditions:
   Wavelength : .....546 nm. (540-550)
   Cuvette: .....1 cm light path
   Constant temperature.....37°C
2. Mix 10 µl sample with 360 µl R1 and incubate at 37°C for 3 minutes.
3. Add 180 µl R2 into cuvette, mix and start stopwatch.
4. Read the absorbance (A1) after 300 seconds and after 180 seconds read the absorbance (A2).
5. Calculate ΔA = A2 - A1.

Calculation

$$\text{ADA (U/L)} = \frac{\Delta A_{Sample}}{\Delta A_{Calibrator}} \times \text{Calibrator value}$$

Quality Control:

Control sera are recommended to monitor the performance of assay procedures.
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference Values

Serum/Plasma		:	0 - 22 U/L
CSF	Normal	:	<10 U/L
	Positive	:	>10 U/L
Pleural, Pericardial & Ascitic Fluids:			
	Normal	:	<40 U/L
	Suspect	:	>40 U/L to <60 U/L
	Positive	:	>60 U/L

These values are for orientation purpose.
Each laboratory should establish its own reference range.

Waste Management

Please refer to local legal requirements.

Reagent Performance

1. **Linearity limit:** The assay is linear up to ADA of 200 U/L. If the results obtained were greater than linearity limit, dilute the sample ½ with NaCl 9 g/L and multiply the result by 2.
2. **Detection limit:** The minimum detectable concentration of ADA with an acceptable level of precision was determined as 1 U/L.

Precision

Intra-assay precision Within run (n=20)	Mean (U/L)	SD (U/L)	CV (%)
Sample 1	131	1.56	1.196
Sample 2	48.48	0.615	1.27

Inter-assay precision Run to run (n=20)	Mean (U/L)	SD (U/L)	CV (%)
Sample 1	19.60	0.737	3.76

Accurancy : Results obtained using the above reagents (y) did not show systematic differences when compared with other commercial reagents (x). The results obtained were the following:
Correlation coefficient (r) = 0.997
Regression equation (y) = 0.998 x + 1.836
The results of the performance characteristics depend on the analyzer used.

Interferences :

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

Applications for automatic analysers are available on request.

Assay Parameters For Photometers

Mode	Fixed Time
Wavelength 1	546
Sample Volume (µl)	10
Reagent 1 Volume (µl)	360
Reagent 2 Volume (µl)	180
Lag time (sec.)	300
Read time (sec.)	180
Reaction temperature (°C)	37
Reaction direction	Increasing
Normal High (U/L)	22
Linearity Low (U/L)	1
Linearity High (U/L)	200
Calibrator Activity	See on vial label
Blank with	Water
Units	U/L

Notes

Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

References

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

Symbols Used On Labels

REF	Catalogue Number		Manufacturer
	See Instruction for Use	LOT	Lot Number
CONT	Content		Storage Temperature
	Expiry Date	IVD	In Vitro Diagnostics

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