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Adenosine Deaminase Assay Kit

Configuration

The Diazyme Adenosine Deaminase reagent is provided in bulk and the following kit configuration:

REF	Kit Size
DZ117A-K	R1: 1 x 50 mL R2: 1 x 25 mL

Intended Use

Adenosine Deaminase (ADA) Assay Kit is for determination of ADA activity in serum, plasma, pleural fluid, and cerebrospinal fluid samples.

Background

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. Published literature states that elevated serum ADA activity has been observed in patients with acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, liver cirrhosis, viral hepatitis and hepatoma.[1,2] Increased ADA activity was also observed in patients with tuberculous effusions.[3] These reports state that 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 and may also be useful in the diagnostics of tuberculous pleuritis.[3]

Assay Principle

The Diazyme 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 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.

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

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

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

$$2H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{POD}} 4H_2O + \text{Quinone dye } (\lambda \text{ max } 556\text{nm})$$

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

Reagent – Working Solutions

Reagent 1	
Tris HCl, pH 8.0	50 mM
4-AA	2 mM
PNP	0.1 U/mL
XOD	0.2 U/mL
Peroxidase	0.6 U/mL
Stabilizers	
Reagent 2	
Tris-HCl, pH 4.0	50 mM
Adenosine	10 mM
EHSPT	2 mM

Precautions

1. EU: CE IVDD
2. R1 is light-sensitive and should be stored in a dark place.
3. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
4. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
5. The reagents contain < 0.1% sodium azide, NaN_3, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.
6. Do not use the reagents after the expiration date labeled on the outer box.
7. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

Reagent Handling

ADA REAGENT comes in a liquid two-reagent system, ready-to-use for both manual method and automated chemistry analyzers (kinetics). ADA CONTROL and CALIBRATOR are in lyophilized form, and need to be reconstituted with 1.0 mL of DI water before use. The reconstituted CONTROLS and CALIBRATOR are stable for 1 week at 2-8°C. CONTROLS and CALIBRATOR sold separately.

Reagent Stability and Storage

REAGENT are stable until their expiration date when stored at 2-8°C.

Specimen Collection and Preparation

Serum, heparinized plasma, pleural fluid, or cerebrospinal fluid 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 4°C[11]. Pleural fluid should be collected in a sterile or heparinized tube and processed within 2 hours at room temperature or stored at 4°C or -20°C for 2 days and up to 2.5 years at -80°C.[7,8,9] Cerebrospinal fluid (CSF) should be clear and collected in a sterile tube without anticoagulant. ADA is stable in CSF for 24 hours at 25ºC, 7 days at 4ºC and 3 months at -20ºC.[10]

Materials Provided

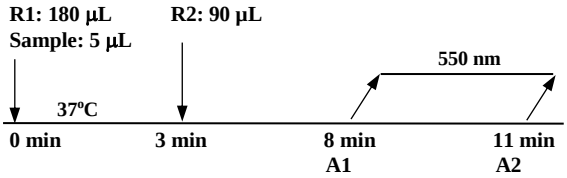
See "Reagent – Working Solutions" section for REAGENT.

Materials Required but not Provided

- Any instrument with temperature control of 37 ± 0.5°C that is capable of reading absorbance accurately at 540nm – 550nm may be used
- Controls for validating the performance of the Diazyme Adenosine Deaminase Assay Kit (REF DZ117A-CON)
- Calibrators for the Diazyme Adenosine Deaminase Assay Kit are provided separately (REF DZ117A-CAL)
- 0.9% Saline is needed as CALIBRATOR 0
- General laboratory equipment

Assay Procedure

Test Scheme for Chemistry Analyzers



Application sheets for use of Diazyme Adenosine Deaminase Assay on automated clinical chemistry analyzers are available upon request. Please call 858-455-4768 or email: support@diazyme.com.

Calibration

0.9% saline and the Diazyme Adenosine Deaminase Calibrator (REF DZ117A-CAL) are needed for calibration. The lot specific CALIBRATOR values are stated in the Certificate of Analysis.

Quality Control

We recommend that each laboratory use the Diazyme Adenosine Deaminase Control Set, listed under Materials Required section, to validate the performance of ADA reagents. The Diazyme ADA Control Set is available from Diazyme Laboratories (REF DZ117A-CON). The CONTROL interval and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Each laboratory should follow federal, state, and local guidelines for testing QC material.

Results

The ADA results are printed out in U/L. Literature cites ADA activity tests in serum samples to be in the range of 0-15 U/L[1-4]. Literature citations show that for pleural fluid, values were found to be in the range of 0-30 U/L, and for cerebrospinal fluid (CSF), values were found to be in the range of 0-9 U/L.[4,6]

Limitations

If the sample ADA activity is greater than 200 U/L, the sample should be diluted with saline before measurement. The result should be multiplied by the dilution factor. Assay is specific for ADA and has no detectable reaction with other nucleosides. The reagent solution should be clear. If turbid, the reagent may have deteriorated.

Analytical Characteristics[5]

Results from individual laboratories may vary.

Precision

The precision of the Diazyme Adenosine Deaminase Assay was evaluated on the Cobas Mira instrument according to a modified Clinical Laboratory Standards Institute EP5-A guideline. In the study, two serum specimens containing 11 U/L and 30 U/L ADA were tested with 2 runs per day with duplicates over 15 working days.

	Within Run Precision		Run to Run Precision	
	11 U/L	30 U/L	11 U/L	30 U/L
No. of Data Points	60	60	60	60
Mean (U/L)	11.1	30.6	11.1	30.6
SD	0.16	0.42	0.21	0.56
C_V%	1.4	1.4	1.9	1.8

Linearity

The linearity of the procedure is from 0 – 200 U/L.

Interference

Assay is not affected by serum bilirubin up to 30 mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 750 mg/dL, and ascorbic acid up to 4 mg/dL.

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