ADENOSINE DEAMINASE ASSAY (ADA)
Dual Vial Liquid Stable Assay

Convenient
• Stable liquid stable format requires no reagent preparation
• Lyophilized calibrator included with the kit *
• High and low controls available

Excellent Performance
• Excellent precision with CV’s of less than 5%
• Extended linearity from 0 – 200 U/L
• Average recovery of 99.7%

Excellent Reagent Stability
• Three month on-board stability
• 12-month kit stability

Specific
• Highly specific for ADA and has no detectable reaction with other nucleosides
• 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

Flexibility
• Requires as little as 5 µL ideal for pediatric, veterinary and research applications**
• Ability to test serum, plasma, CSF and pleural effusions **
• Automated and manual assay parameters available

*Packaged separately
**Assay is for research only in the United States.
**ADENOSINE DEAMINASE ASSAY (ADA)**

**SUMMARY OF PERFORMANCE**

**Background**

ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. The enzyme is widely distributed in human tissues, and is 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 \( \gamma \)-GT (GGT) tests. ADA assay may also be useful in the diagnostics of tuberculous pleuritis.

**Assay Method**

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 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.

**Performance**

**Method Comparison**

To demonstrate accuracy, the Diazyme Adenosine Deaminase Enzymatic Assay was tested with individual serum samples with comparison to results obtained by an accredited reference clinical laboratory using their analyte specific reagents based upon the reference method for ADA activity in serum.

The individual patient serum or plasma samples used for this study were from a certified commercial source. A small sample of ten (10) patient samples ranging from 13 - 48 U/L were tested which gave a correlation coefficient of 0.966. This study yielded a linear regression equation of \(y = 0.9662x - 0.02\) U/L.

**Precision**

The precision of the Diazyme Adenosine Deaminase Enzymatic Assay was evaluated according to a modified Clinical and Laboratory Standards Institute (formerly NCCLS) EP5-A protocol. In the study, two specimens containing 11.0 ± 2.75 and 30.0 ± 5.4 U/L Adenosine Deaminase were tested with 2 runs per day with duplicates over 15 working days.

**Analytical Sensitivity**

To demonstrate the limit of detection (LOD) of Diazyme Adenosine Deaminase Enzymatic Assay, Adenosine Deaminase zero calibrator was tested on 12 replicates on Cobas Mira. The LOD is defined as mean + 3SD. Based on these studies the LOD = 0.003 + 0.03 = 0.033 U/L Adenosine Deaminase.

**Linearity**

Ten levels of samples with ADA activity were prepared by serially diluting a serum control containing 200 U/L Adenosine Deaminase with distilled H\(_2\)O. Based on this study the assay is linear to 200 U/L.

**Interference**

To determine the level of interference from the substances normally present in the serum, Diazyme Adenosine Deaminase Enzymatic Assay was evaluated by running three (3) replicates each of a control sample in the absence and presence of various potential interference substances at indicated concentrations. Assay is not affected by interfering substances such as serum bilirubin up to 30 mg/dL, hemoglobin up to 500 mg/dL, triglycerides up to 500 mg/dL, ascorbic acid up to 20 mg/dL, and ammonia up to 800 µmol.

**Within-run Precision**

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean (U/L)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>11.11</td>
<td>0.16</td>
<td>1.47</td>
</tr>
<tr>
<td>Level 2</td>
<td>30.74</td>
<td>0.45</td>
<td>1.45</td>
</tr>
</tbody>
</table>

**Between-Run Precision**

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean (U/L)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>9.63</td>
<td>0.47</td>
<td>4.90</td>
</tr>
<tr>
<td>Level 2</td>
<td>29.62</td>
<td>0.59</td>
<td>2.00</td>
</tr>
</tbody>
</table>

**DIAZYME LABORATORIES**

12889 Gregg Court, Poway, CA 92064  PO Box 85608, San Diego, CA 92186

Tel: 858-455-4768    888-DIAZYME    WWW.DIAZYME.COM    SALES@DIAZYME.COM
Adenosine Deaminase Assay Kit

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

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Catalog No.</th>
<th>Kit Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal</td>
<td>DZ117A-K</td>
<td>R1: 1 x 50 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R2: 1 x 25 mL</td>
</tr>
</tbody>
</table>

Note: Calibrators sold separately

Intended Use
Adenosine deaminase (ADA) assay kit is for determination of ADA activity in human serum and plasma samples. For investigational use in the USA only.

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.1,2 Increased ADA activity was also observed in patients with tuberculous effusions.3 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.3

Assay 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-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.

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.

Reagent Composition

<table>
<thead>
<tr>
<th>Active Ingredients</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris HCl, pH 8.0</td>
<td>50 mM</td>
</tr>
<tr>
<td>4-AA</td>
<td>2 mM</td>
</tr>
<tr>
<td>PNP</td>
<td>0.1 U/mL</td>
</tr>
<tr>
<td>XOD</td>
<td>0.2 U/mL</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>0.6 U/mL</td>
</tr>
<tr>
<td>Stabilizers</td>
<td></td>
</tr>
<tr>
<td>Tris-HCl, pH 4.0</td>
<td>50 mM</td>
</tr>
<tr>
<td>Adenosine</td>
<td>10 mM</td>
</tr>
<tr>
<td>EHSPT</td>
<td>2 mM</td>
</tr>
<tr>
<td>ADA Control</td>
<td></td>
</tr>
</tbody>
</table>

Reagent Preparation
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 ADA controls and calibrator are stable for 1 week at 2-8°C. Control and calibrator sold separately.

Reagent Stability and Storage
Reagents are stable until their expiration date when stored at 2-8°C.

Specimen Collection and Handling
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 2-4°C.

Precautions
1. Reagent R1 is light-sensitive. Store in a dark place.
2. 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).
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
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.

**Assay Procedure**

**Test Scheme for Chemistry Analyzers**

<table>
<thead>
<tr>
<th>R1: 180 µL</th>
<th>R2: 90 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample: 5 µL</td>
<td>37°C</td>
</tr>
</tbody>
</table>

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**

A single calibrator, along with 0.9% saline as a zero reference, should be used as directed to calibrate the procedure.

**Quality Control**

Diazyme recommends that each laboratory use ADA controls to validate the performance of ADA reagents. ADA controls are available from Diazyme Laboratories (Cat. # DZ117A-Con). If the results from the control falls outside the acceptable limits, the test should not be performed. We recommend that your quality control testing follows federal, state, and local guidelines.

**Results**

The ADA results are printed out in U/L.

**Reference Range**

We have tested ADA activity in 60 healthy human serum samples and found to be in the range of 0-15 U/L. For pleural fluid, values were found to be in the range of 0-30 U/L, and for C.S.F., values were found to be in the range of 0-9 U/L. It is recommended that each laboratory establish its own range of reference values.

**Limitations**

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.

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.

**Performance Characteristics**

**Precision**

The precision of the Diazyme Adenosine Deaminase Assay was evaluated on the Cobas Mira instrument according to a modified Clinical Laboratory Standards Institute EPS-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.

<table>
<thead>
<tr>
<th></th>
<th>Within Run Precision</th>
<th>Run to Run Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Data Points</td>
<td>11 U/L</td>
<td>30 U/L</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>11.11</td>
<td>30.74</td>
</tr>
<tr>
<td>SD</td>
<td>0.16</td>
<td>0.45</td>
</tr>
<tr>
<td>CV%</td>
<td>1.47%</td>
<td>1.45%</td>
</tr>
</tbody>
</table>

**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.

**References**