

Diagnostic reagent for quantitative in vitro determination of adenosine deaminase (ADA) in human serum, plasma, pleural fluid, and cerebrospinal fluid on photometric systems

Test parameters

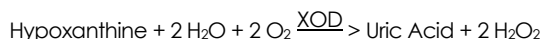
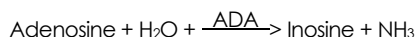
| | |
|--------------|---|
| Method: | Colorimetric, 2-point kinetic, increasing reaction, enzymatic |
| Wavelength: | 550 nm |
| Temperature: | 37 °C |
| Sample: | Serum, heparinized plasma, pleural fluid, cerebrospinal fluid |
| Linearity: | up to 200 U/L |
| Sensitivity: | Limit of detection: 0.03 U/L |

Summary

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].

TEST 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.



REAGENT COMPOSITION

| COMPONENTS | CONCENTRATION |
|-------------------|---------------|
| Reagent 1: | |
| Tris HCl, pH 8.0 | 50 mmol/L |
| 4-Aminoantipyrine | 2 mmol/L |
| PNP | 0.1 kU/L |
| XOD | 0.2 kU/L |
| Peroxidase | 0.6 kU/L |

Reagent 2:

| | |
|------------------|-----------|
| Tris HCl, pH 4.0 | 50 mmol/L |
| Adenosine | 10 mmol/L |
| EHSPT | 2 mmol/L |

REAGENT PREPARATION

The reagents are ready to use.

REAGENT STABILITY AND STORAGE

Conditions: R1 is light sensitive. Protect from light!
Store in a dark place.
Close immediately after use.
Do not freeze the reagents!
Avoid contamination.

Stability: at 2 – 8 °C up to the expiration date

The reagent should be clear. If turbid, the reagent may have deteriorated.

SAMPLE PREPARATION

Ideally, venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant. Prompt separation from cells or clot is recommended.

Pleural fluid should be collected in a sterile or heparinized tube resp.

Cerebrospinal fluid (CSF) should be clear and collected in a sterile tube without anticoagulant.

SAMPLE STABILITY AND STORAGE

| | | |
|---------------------------------|-------------|-----------------|
| Serum/plasma [3]: | at 2 – 4 °C | 1 week |
| Pleural fluid [6,7,8]: | room temp. | 2 hours |
| | at 2 – 4 °C | 2 days |
| | at -20 °C | 2 days |
| | at -80 °C | up to 2.5 years |
| Cerebrospinal fluid [9]: | at 25 °C | 24 hours |
| | at 4 °C | 7 days |
| | at -20 °C | 3 months |

Keep serum/plasma tightly stoppered.

Materials required but not provided

NaCl solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

| Pipette into test tubes | Blank | Calibrator | Sample |
|--|--------|------------|--------|
| Reagent 1 | 900 µL | 900 µL | 900 µL |
| Sample or Std./Cal. | - | 25 µL | 25 µL |
| Distilled water | 25 µL | - | - |
| Mix. Incubate for 3 min. at 37°C. Then add: | | | |
| Reagent 2 | 450 µL | 450 µL | 450 µL |
| Mix. Incubate 5 min. at 37 °C and read A1 against reagent blank. Incubate for exactly 3 min. at 37 °C and read A2 against reagent blank. Calculate: ΔA = (A2 - A1) | | | |

CALCULATION

$$\text{ADA [U/L]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}} \times \text{Conc. Cal [U/L]}$$

REFERENCE RANGE *

| | |
|-----------------------------|-------------------|
| Serum [1-4]: | 0 – 15 U/L |
| Pleural fluid [4,5]: | 0 – 30 U/L |
| CSF [4,5]: | 0 – 9 U/L |



* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY / MEASURING RANGE

The test has been developed to determine ADA concentrations within a measuring range from 0.03 – 200 U/L. When values exceed this range, samples should be diluted with NaCl solution (9 g/L) and re-assayed multiplying the result by the dilution factor.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 0.03 U/L

PRECISION (at 37°C)

| Within run, n = 30 | Mean [U/L] | SD [U/L] | CV [%] |
|--------------------|------------|----------|--------|
| Sample 1 | 11.1 | 0.16 | 1.47 |
| Sample 3 | 30.7 | 0.45 | 1.45 |

| Run to run, n = 30 | Mean [U/L] | SD [U/L] | CV [%] |
|--------------------|------------|----------|--------|
| Sample 1 | 9.63 | 0.47 | 4.90 |
| Sample 3 | 29.6 | 0.59 | 2.00 |

SPECIFICITY/INTERFERENCES

no interference up to:

| | |
|---------------|-----------|
| Ascorbic acid | 4 mg/dL |
| Bilirubin | 30 mg/dL |
| Hemoglobin | 200 mg/dL |
| Triglycerides | 750 mg/dL |

Calibration

The assay requires the use of an ADA calibrator. We recommend our **ADA Calibrator** and 0.9% saline as a zero calibrator.

QUALITY CONTROL

All controls with ADA values determined by this method can be used. We recommend our **ADA Control Set**. Each laboratory should establish corrective action in case of deviations in control recovery.

WARNINGS AND PRECAUTIONS

- The reagents contain < 0.1% sodium azide 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.
- Avoid ingestion and contact with skin and eyes.
- Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
- For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
- For professional use only!

WASTE MANAGEMENT

Please refer to local requirements.

References

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Symbols on labels and packaging

 = In vitro diagnostic medical device

 = Catalog Number

 = Lot Number

 = Manufacturer

 = Expiration date

 = Temperature limitation

 = Instruction for use

