OBio...
Supporting Care to Cure
A Division of Orchard Medical

# ADENOSINE DEAMINASE (ADA)

## Order Information

| Cat. No. | Kit Configuration |
|---|---|
| OAR1010 | Reagent 1: 1 x 20 mL<br>Reagent 2: 1 x 10 mL<br>Calibrator: 1 x 1 mL |

## Summary

ADA is an enzyme catalyzing the de-amination 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.

## Method

Colorimetric-Kinetic 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 ($H_2O_2$) 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.

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

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

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

$$2H_2O_2 + \text{4-AA} + \text{TOOS} \xrightarrow{\text{POD}} 4H_2O + \text{Quinone dye}$$

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

## Reagents

Storage Instructions and Reagent Stability

Reagent is stable up to the end of the indicated month of expiry, if stored at 2°– 8°C, protected from light and contamination is avoided. Do not freeze the reagents!
Reagent 1: Enzyme
Solution Reagent 2:
Substrate Solution
Calibrator: ADA Calibrator (ADA value on label)

## Composition

Reagent: Tris-HCl (pH: 8.0); 4-AA: 2mM; PNP: 0.1 U/L; XO: 0.2 U/L;
POD: 0.6 U/L; Adenosine: 10mM; preservative.

## Warnings and Precautions

1. Cuvette and other glassware must be cleaned thoroughly after being used for other assays. In case of automated measurement refer to the instrument manual for special washing programs.
2. In very rare cases, samples of patient's with gammopathy might give falsified results.
3. 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.

## Waste Management

Please refer to local legal requirements.

## Reagent Preparation

The reagents are ready to use. ADA Calibrator is in lyophilized form and need to be reconstituted with 1.0 mL of distilled water before use.
Calibrator after reconstitution is stable till expiry if stored at –20° C and for 1 week at 2° – 8°C, if protected from light and contamination is avoided.

## Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

## Specimen

Cerebrospinal fluid or Serum, heparin plasma or EDTA plasma separate at the latest 1h after blood collection from cellular contents.

| | | |
|---|---|---|
| 7 days | at | 2° –8°C |
| 30 days | at | –20°C |

Only freeze once! Discard contaminated specimens.

## Assay Procedure

| | |
|---|---|
| Wavelength | 546 nm (540 – 550 nm) |
| Optical path | 10 mm |
| Temperature | 37°C |

| | For Sample/Calibrator |
|---|---|
| Reagent 1 | 360 µL |
| Sample/ Calibrator | 10 µL |
| Mix and incubate at 37°C for 3 minutes, then add Reagent 2 | |
| Reagent 2 | 180 µL |
| Mix and Incubate for 5 minutes and read absorbance (A1) and again after 3 minutes (A2). | |

## Calculations

$\Delta A = A2 - A1$

$$\text{U/L of ADA in the sample} = \frac{\text{Sample } \Delta A/\text{min}}{\text{Calibrator } \Delta A/\text{min}} \times \text{Calibrator value}$$

Quality Controls: For internal quality control any normal and abnormal controls should be assayed with each batch of samples.
Each laboratory should establish corrective action in case of deviations in control recovery.

## Performance Characteristics Measuring Range

**The test has been developed determine ADA within a measuring range from 1 – 200 U/L. If such value is exceeded the sample should be diluted 1+1 with NaCl solution (9 g/L) and results multiplied by 2.**

## Interferences

**No interference was observed by, Ascorbic acid up to 30 mg/dL, Bilirubin up to 40 mg/dL and Triglycerides up to 1000 mg/dL.**

## Sensitivity/Limit of Detection

**The lower limit of detection is 1 U/L.**

## Linearity

**The higher limit of detection is 200 U/L.**

## Precision

| Intra-assay n = 20 | Mean (U/L) | SD (U/L) | CV (%) |
|---|---|---|---|
| Sample 1 | 44.60 | 0.73 | 1.64 |
| Sample 2 | 18.20 | 0.07 | 0.38 |

| Inter-assay n = 20 | Mean (U/L) | SD (U/L) | CV (%) |
|---|---|---|---|
| Sample 1 | 35.59 | 0.09 | 0.26 |
| Sample 2 | 16.30 | 0.06 | 0.39 |

## Method Comparison

**A comparison of Nucleus Diagnosys ADA (y) with a commercially available test (x) using 15 samples gave following results:**
**$y = 0.976x + 0.913$; $R^2 = 0.997$**

## Reference Range

**Serum/Plasma: 0 – 15 U/L CSF:- Normal : 0 – 10 U/L**
**Pleural Fluids:- Normal : 0 – 30 U/L**

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

## Literature


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