Catalog #80249

V.2

Crystal Chem

Adenosine Deaminase Assay (ADA) Kit Instructions

For the quantitative determination of adenosine deaminase in serum or plasma

Catalog #80249
120 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Adenosine Deaminase (ADA) kit is for the quantitative determination of adenosine deaminase activity in serum or plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

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. Increased ADA activity was also observed in patients with tuberculous effusions.

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.

C. Principle of the Assay

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

D. Kit Storage

1. Upon receipt of the ADA Kit, store the reagents at 2-8°C (do not freeze the kit or hold it at temperatures above 25°C). **Calibrator (packaged separately) should be stored at -20°C.**
3. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
CC1	Reagent CC1 (liquid)	1 X 25 mL
CC2	Reagent CC2 (liquid)	1 X 12.5 mL
CAL1	Calibrator 1 (lyophilized)	1 X 1 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Clean glass tubes and test tube racks
Incubator (37ºC)
Distilled water
Spectrophotometer (should read A_{550} values)
0.9% Saline

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human or bovine sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Reagent CC1 is light-sensitive. Store in a dark place. Do not let bottles remain open. Keep containers tightly closed.
4. Reagents CC1 and CC2 contain < 0.1% sodium azide (NaN_3) as a preservative, which 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.
5. Do not use the reagents after the expiration date.

G. Maximizing Kit Performance

1. Given the small sample volumes required (5 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the glass tubes from drying out and to get the best results, samples and reagents should be dispensed quickly into the tubes.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

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 2–8°C.

I. Assay Procedure

I.1. Preparation of reagents

All reagents are provided ready-to-use and should be brought to room temperature for at least 30 minutes prior to use. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling

I.2. Preparation of samples, calibrators, and controls

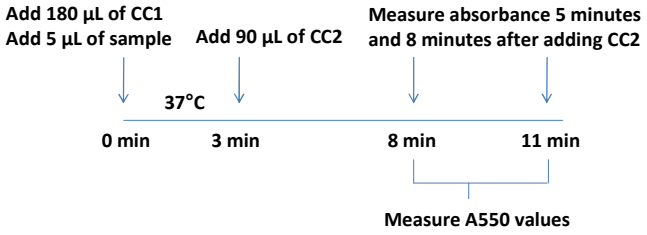
1. Allow calibrator to thaw and reconstitute the calibrator with 1 mL of deionized water. To ensure complete reconstitution, equilibrate vials at room temperature for 30 minutes before first use.
   Note: Reconstituted calibrators are stable for 7 days when capped tightly and stored at 2-8°C. In addition to running the calibrator provided, the assay requires running a blank calibrator. 0.9% saline should be used for running the blank calibrator. Optional controls are sold separately (Cat# 80243). Controls should be reconstituted with 1 mL of deionized water as well.
2. Bring all samples, calibrators, and controls to room temperature.

I.3. Assay procedure

The procedure below reflects a manual procedure performed using a glass tube with a spectrophotometer. The assay can also be adopted to work on a microplate and various automated analyzers. Please contact Crystal Chem for more information.

1. Add 180 µL of Reagent CC1 and 5 µL of sample, calibrator, or control into a clean glass tube and mix well by repeated pipetting.
2. Place glass tube in incubator (37ºC) and allow tube to equilibrate to 37ºC over 3 minutes.
3. Pipette 90 µL of Reagent CC2 into the glass tube and mix well by repeated pipetting. Start timer immediately upon addition of Reagent CC2.
   Note: *The accuracy of the assay is based on measuring the change in absorbance at 5 mins and 8 mins after the addition of Reagent CC2. Slight variations in the timing of the readings (ie. 4.5 mins and 8.5 mins) should not affect the results as long as the timing of the readings is consistent for both the calibrators and samples. Said another way, it is important that CC2 be added to the calibrators and samples at the same time and readings for both calibrators and samples be taken at the same time to obtain comparable absorbance readings.*
4. Measure absorbance using a spectrophotometer (measure A_{550} values) 5 mins and 8 mins after the addition of Reagent CC2.

Figure 1. Summary of assay procedure



I.4. Determining the ADA activity

1. Calculate the change in absorbance ΔA (8 mins ~ 5 mins)

   $\Delta A = (OD_{550nm,\ 8\ mins}) - (OD_{550nm,\ 5\ mins})$

2. Using linear graph paper, construct the ADA calibration curve by plotting the mean change in absorbance value for the calibrator (incl. blank) on the Y axis versus the corresponding ADA activity on the X axis.
   Note: *Calibrator value varies per lot and should be obtained from the calibrator label.*
3. ADA activity in the samples are interpolated using the calibration curve and mean change in absorbance values for each sample. This interpolation can be simplified using Equation 1 below. The ADA activity is expressed as U/L.
   Note: *Samples with high ADA activity (200.0 U/L or higher) should be diluted with 0.9% saline and rerun.*

Equation 1. Calculation of ADA activity

ADA Activity =

[(sample ΔA550 –blank ΔA550) / (cal Δ A550 – blank ΔA550)] × cal conc.

J. Performance characteristics

J.1. Assay range

The Adenosine Deaminase assay has a linear range from 0 - 200.0 U/L.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

Warranty

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