Adenosine Deaminase, ADA Assay Kit (Colorimetric Method)

Catalog No.: abx090675

Size: 96 tests (80 samples)

Detection Range: 0.03 U/L – 99 U/L

Sensitivity: 0.03 U/L

Storage: Store all components at 2 – 8°C for up to 6 months.

Application: For detection and quantification of ADA activity in serum, plasma, tissue and other biological fluids.

Introduction

Adenosine deaminase (also known as adenosine aminohydrolase, or ADA) is an enzyme involved in purine metabolism. It is needed for the breakdown of adenosine from food and for the turnover of nucleic acids in tissues. It is found in all tissues, occurs in large amounts in T-lymphocytes and, at the time of weaning, in gastrointestinal tissues. ADA is considered one of the key enzymes of purine metabolism. The enzyme has been found in bacteria, plants, invertebrates, vertebrates, and mammals, with high conservation of amino acid sequence. The high degree of amino acid sequence conservation suggests the crucial nature of ADA in the purine salvage pathway. Primarily, ADA in humans is involved in the development and maintenance of the immune system. However, ADA association has also been observed with epithelial cell differentiation, neurotransmission, and gestation maintenance.

Abbexa's ADA Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Adenosine deaminase (ADA) activity. ADA catalyzes the hydrolysis of adenosine to produce inosine and ammonia, and the activity of ADA can be calculated by red coloration of the ammonia. The absorbance should be measured at 550 nm. The intensity of the red color is proportional to the activity of the ADA enzyme(s), which can then be calculated.

Kit components

1. 96-well microplate
2. Working solution: 20 ml
3. Chromogenic agent: 10 ml
4. Standard (1 mmol/L): 2 ml × 2 vials
5. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (550 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator or Sonicating water bath
7. Centrifuge
8. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 x g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml Normal saline. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Mouse Serum	1
Human Serum	1
10% Rat Kidney Tissue Homogenate	1
10% Rat Heart Tissue Homogenate	2-3
10% Rat Spleen Tissue Homogenate	2-3
10% Mouse Liver Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- Bring all reagents to room temperature before use.

B. Assay Procedure

1. **Standard curve preparation:** Label 8 tubes with 0, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8 and 1.0 mmol/L. Dilute the 1 mmol/L standard with double distilled water to concentrations of 0.2, 0.4, 0.5, 0.6, 0.7, 0.8 and 1.0 mmol/L. The double distilled water itself serves as the 0 mmol/L (blank) standard.

2. Chromogenic Reaction:

2.1. Set the Standard and Sample wells on the well-plate.
2.2. Add 10 µl of prepared standards to the Standard wells.
2.3. Add 10 µl of sample to the Sample wells.
2.4. Add 180 µl of Working solution to each well.
2.5. Add 90 µl of Chromogenic agent to each well.
2.6. Incubate at 37°C for 7 minutes.
2.7. Measure and record the OD values of Sample wells at 550 nm with a microplate reader as A_1.
2.8. Incubate at 37°C for 10 minutes.
2.9. Measure and record the OD values of Standard and Sample wells at 550 nm with a microplate reader as A_2.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{550} of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve ($y = ax + b$). The ADA activity of the samples can be interpolated from the standard curve.

1. Serum and plasma samples:

One unit of ADA activity is defined as the quantity of ADA in 1 L of sample that catalyzes the production of 1 µmol/L hypoxanthine riboside at 37°C per minute.

$$\text{ADA activity (U/L)} = \frac{A_2 - A_1 - b}{a} \times \frac{1000^*}{T} \times f$$

2. Tissues samples:

One unit of ADA activity is defined as the quantity of ADA in 1 g of tissue protein that catalyzes the production of 1 μmol/L hypoxanthine riboside at 37°C per minute.

$$\text{ADA activity (U/g protein)} = \frac{A_2 - A_1 - b}{a} \times \frac{1000^*}{T} \times \frac{f}{C_P}$$

where:

a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
A_1	OD value of the sample after first incubation for 7 minutes
A_2	OD value of the sample after second incubation for 10 minutes
T	second incubation time, 10 minutes
1000^*	1 mmol=1000 μmol
f	dilution factor of the sample before carrying out the assay
C_P	concentration of protein in sample (g protein/L)