Adenosine Deaminase, ADA Assay Kit (Colorimetric Method)

Catalog No.: abx090675
Size: 100 T
Range: 0.4-200 U/ml
Detection limit: 0.4 U/ml
Application: For detection the activity of ADA in serum, plasma or tissue homogenate.
Storage and Expiration: Store at 2-8 °C for 6 months. Avoid exposure to light.

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.

ADA catalyzes the hydrolysis of adenosine to produce inosine and ammonia, and the activity of ADA can be calculated by coloration of the ammonia.

Kit components

1. Reagent A: 6 Reagent A vials (lyophilized) and 6 Reagent A Diluent Buffer Solutions (5 ml each)
2. Reagent B: 2 Reagent B vials (lyophilized) and 2 Reagent B Diluent Buffer Solutions (100 ml each)
3. Reagent C: 2 Reagent C Storage Buffer (2 ml each) and 2 Reagent C Diluent Buffer (100 ml each)
4. Standard: 1 vial (1 ml)

Material Required But Not Provided

1. 37 °C incubator
2. Microplate reader or spectrophotometer (wavelength: 640 nm)
3. Precise pipette and disposable pipette tips
4. Centrifuge
5. Vortex mixer

Protocol

A. Preparation of sample

- **Serum and plasma:** Can be analyzed directly.
- **Tissue homogenates:** Cut and weigh samples. Add normal saline (NS) and homogenize thoroughly. Centrifuge at 2000-3000 rpm for 20 min and collect supernatant.

Note: This kit just can detect the 10% tissue homogenate, after coloration, centrifuge at 3500 rpm for 10 min, then, read the O.D. value.

B. Reagent Preparation

1. Reagent A: Reconstitute 1 Reagent A vial with 1 Reagent A Diluent Buffer Solution bottle. The volume should be 5 ml. Prepare ideally 2 hours before use, store at 4°C.
2. Reagent B: Reconstitute 1 Reagent B vial with 1 Reagent B Diluent Buffer Solution bottle. The volume should be 100 ml. Store at 4°C in the dark.
3. Reagent C: Dilute 2 ml Reagent C Storage Buffer with 100 ml Reagent C Diluent Buffer Solution. The total volume should be 102 ml. Store at 4°C in the dark.
4. Standard: Add 0.25 ml of 1 mg/ml standard into 10 ml of double distilled water to prepare a 25 µg/ml standard.

C. Assay Procedure

1. Set Blank, Standard, Testing and Control Vials.
2. Mix the kit components: Double distilled water, Standard, Reagent A and the testing sample thoroughly, please see the detailed volume of each reagent as below.

	Blank Vial (ml)	Standard Vial (ml)	Testing Vial (ml)	Control Vial (ml)
Double distilled water	0.02			
Standard		0.02		
Testing Sample			0.02	0.02
Reagent A	0.25	0.25	0.25	

3. Mix the above thoroughly and incubate at 37°C for 60 min.
4. Add 1.5 ml of Reagent B & C into each vial, and 0.25 ml of Reagent A into the Control Vial.

	Blank Vial (ml)	Standard Vial (ml)	Testing Vial (ml)	Control Vial (ml)
Reagent A				0.25
Reagent B	1.5	1.5	1.5	1.5
Reagent C	1.5	1.5	1.5	1.5

5. Vortex to mix thoroughly and incubate at 37 °C for 30 min, place at room temperature for 10 min.
6. Read the O.D. absorbance of each vial at 640 nm, 1 cm optical path in a spectrophotometer.

D. Calculation

Serum & Plasma ADA activity (U/ml)

= (O.D.640 of each well - O.D.640 of Control Vial) / (O.D.640 of Standard Vial - O.D.640 of Blank Vial) x Concentration of the Standard (25 µg/ml)

Tissue homogenate NO activity (U/mgprot)

= (O.D.640 of each well - O.D.640 of Control Vial) / (O.D.640 of Standard Vial - O.D.640 of Blank Vial) x Concentration of the Standard (25 µg/ml) / target protein concentration of the testing sample (mgprot/ml)