CREATIVE BIOMART®
Assay Kit

Adenosine Deaminase Activity Assay Kit (Colorimetric)

Product Information

Common Name

ADA

Cat.No. Kit-1101

Description

Adenosine Deaminase (ADA) (E.C. 3.5.4.4.) is an enzyme that catalyzes the conversion of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine. Adenosine Deaminase is widely distributed in various tissues and cells. There are two isoforms, ADA1 and ADA2. ADA1 is widely expressed in most cells in the body, particularly in lymphocytes and macrophages. It is present in the cytosol, nucleus and found associated with dipeptidyl peptidase-4 on the cell membrane. ADA2 was first found in the spleen but is predominantly found in the plasma and serum. Increased serum ADA levels are found in certain infectious diseases such as tuberculosis and various liver diseases such as acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis to name a few. Adenosine Deaminase is also a marker for T-lymphocyte proliferation. In ADA Activity Assay, inosine formed from the breakdown of adenosine is converted to uric acid with ADA Convertor and ADA Developer. The uric acid is measured at OD 293 nm. The kit measures total Activity of Adenosine Deaminase with limit of quantification of 1 mU recombinant Adenosine Deaminase.

Applications

Detection of Adenosine Deaminase (ADA) activity

Storage

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

Size

100 assays

Kit Components

ADA Assay Buffer (10x) 25 ml

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ADA Convertor 1 Vial
ADA Developer 1 Vial
ADA Substrate 500 µl
ADA Positive Control 1 Vial
Inosine Standard (10 mM) 100 µl
U.V. transparent plate (96-well) 1

Materials Required but Not Supplied

- 96-well clear plate with flat bottom.
- Microplate reader capable of absorbance measurement
- Protease Inhibitor Cocktail
- Dounce Homogenizer

Compatible Sample Types

Purified recombinant protein
Nuclear Extract
Cell and tissue lysate

Preparation

Reagent Preparation:
ADA Assay Buffer (10x): Make 1x buffer by adding one part 10x Assay Buffer to nine parts deionized water. Store at -20°C or 4°C. Bring to 37°C before use.
ADA Converter and ADA Developer: Reconstitute each with 210 µl 1x ADA Assay Buffer and mix gently by pipetting. Briefly centrifuge to collect the contents in the bottom of the tube. Aliquot and store at -20°C. Avoid repeated freeze/thaw.
ADA Substrate: Aliquot and store at -20°C. Avoid repeated freeze/thaw.
ADA Positive Control: Reconstitute with 25 µl 1x ADA Assay Buffer and mix gently by pipetting. Briefly centrifuge to collect the contents in the bottom of the tube. Aliquot and store at -20°C. Avoid repeated freeze/thaw.

Assay Protocol

1. Sample Preparation: Rinse tissue and transfer ~100 mg of fresh or frozen tissue (stored at -80°C) to

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a pre-chilled homogenizer. Add 300 µl cold 1x ADA Assay Buffer containing protease inhibitor cocktail (not provided) and thoroughly homogenize tissue on ice. Transfer the tissue homogenate to a cold microfuge tube.
To prepare cell extract, add 150-300 µl cold 1x ADA Assay Buffer containing protease inhibitor cocktail (not provided) to 1-5 x 10^6 fresh or frozen cells and pipette several times to disrupt the cells. Transfer cell homogenate including cell debris to a cold microfuge tube and agitate on a rotary shaker at 4°C for at least 15 min.
Centrifuge the tissue or cell homogenate at 16,000 X g, 4°C for 10 min. Transfer the clarified supernatant to a fresh pre-chilled tube & store on ice. Use lysates immediately to assay Adenosine Deaminase activity.
Note: Lysates can be aliquoted and snap frozen in liquid nitrogen before storing at -80°C. Avoid freeze/thaw.
2. Inosine Standard: Dilute Inosine Standard to 1 mM by adding 10 µl of 10 mM Inosine Standard to 90 µl 1x ADA Assay Buffer. Add 0, 2, 4, 6, 8 and 10 µl of diluted 1 mM Inosine Standard into a series of wells in 96-well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well Inosine Standard. Adjust the volume to 50 µl/well with 1x ADA Assay Buffer.
3. Adenosine Deaminase Activity Assay: Add 2-50 µl of sample into desired well(s) in 96-well plate. For Positive Control, use 1-2 µl Positive Control into desired well(s). Make up the volume of samples and Positive Control to 50 µl/well with 1x ADA Assay Buffer. Add 50 µl 1x ADA Assay Buffer to one well as reagent Background Control.
Notes: a. For unknown samples, we suggest doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.
b. Small molecules such as inosine, xanthine and hypoxanthine in the samples will contribute to the background. Remove these molecules by passing through a desalting column or by buffer exchange using a 10kDa spin column. Use this modified sample for the assay. Optional: prepare a parallel sample well as sample background control to ensure that the small molecules are removed by either using a desalting or spin column.
4. Reaction Mix: Prepare enough reagents for the number of assays to be performed. Make 50 µl of Reaction Mix and Background Control Mix containing:

Reaction Mix	Background Control Mix

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1x ADA Assay Buffer 41 µl 46 µl
ADA Convertor 2 µl 2 µl
ADA Developer 2 µl 2 µl
ADA Substrate 5 µl -----
Add 50 µl of Reaction Mix into each sample, reagent background control and Positive Control wells and 50 µl of Background Control mix to Standards and sample background control well(s). Mix well.
5. Measurement: Preincubate at 37°C for five min. and then measure absorbance (OD 293 nm) in kinetic mode for at least thirty min. at 37°C. Choose two time points (T1 & T2) in linear range (can be as short as 2 min.) of plot and obtain corresponding absorbance for sample (AS1 and AS2) and reagent background control (ABG1 and ABG2). Read the Inosine Standard Curve along with the samples.
6. Calculations: Subtract 0 Standard reading from all Standard Readings. Plot the Inosine Standard Curve. Subtract reagent background control reading from sample reading. Apply the ΔOD [(AS2-ABG2) – (AS1-ABG1)] to the Standard Curve to get B nmol of Inosine generated by the sample during the reaction time (ΔT = T2 - T1).
Note: If sample background control reading is significant, subtract sample background control reading from sample reading instead of subtracting reagent background control reading and use this ΔOD to determine B nmol of Inosine generated by the sample during the reaction time (ΔT = T2 - T1).
Sample's ADA Activity =B / (ΔT × µg of protein) x DF = nmol/min/µg = mU/µg
Where:
B is Inosine amount from Standard Curve (nmol).
ΔT is the reaction time (min.)
µg of protein is the amount of protein/well (µg)
DF is the dilution factor of the sample
Sample ADA Activity can also be expressed as U/mg (µmole/min. inosine generated per mg) of protein.
Unit Definition: One unit of Adenosine Deaminase Activity is the amount of enzyme that hydrolyzes adenosine to yield 1.0 µmol of inosine/min. at 37°C.

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