

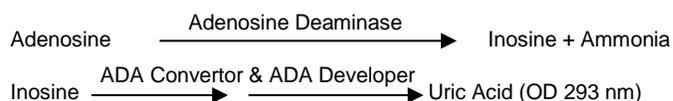
Adenosine Deaminase Activity Assay Kit (Colorimetric)

(Catalog # K321-100; 100 assays; Store at -20°C)

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I. Introduction:

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 BioVision's ADA Activity Assay, inosine formed from the breakdown of adenosine is converted to uric acid with ADA Converter 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.



II. Application:

- Detection of Adenosine Deaminase (ADA) activity

III. Sample Type:

- Purified recombinant protein
- Nuclear Extract
- Cell and tissue lysate

IV. Kit Contents:

Components	K321-100	Cap Code	Part Number
ADA Assay Buffer (10x)	25 ml	WM	K321-100-1
ADA Converter	1 Vial	Green	K321-100-2
ADA Developer	1 Vial	Blue	K321-100-3
ADA Substrate	500 µl	Brown	K321-100-4
ADA Positive Control	1 Vial	Violet	K321-100-5
Inosine Standard (10 mM)	100 µl	Yellow	K321-100-6
U.V. transparent plate (96-well)	1	—	K321-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom.
- Microplate reader capable of absorbance measurement
- Protease Inhibitor Cocktail (Cat. # K271 or its equivalent)
- Dounce Homogenizer (Cat. # 1998 or its equivalent)

VI. Storage Conditions and Reagent Preparation:

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

- 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.

VII. Adenosine Deaminase Activity Assay Protocol:

1. Sample Preparation: Rinse tissue and transfer ~100 mg of fresh or frozen tissue (stored at -80°C) to 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 10⁶ 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 (Cat. # 6564) or by buffer exchange using a 10kDa spin column (Cat # 1997). 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
1x ADA Assay Buffer	41 µl	46 µl
ADA Converter	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 (A_{S1} and A_{S2}) and reagent background control (A_{BG1} and A_{BG2}). 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 [(A_{S2}-A_{BG2}) - (A_{S1}-A_{BG1})] 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).

$$\text{Sample's ADA Activity} = \frac{B}{\Delta T \times \mu\text{g of protein}} \times \text{DF} = \text{nmol/min}/\mu\text{g} = \text{mU}/\mu\text{g}$$

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.

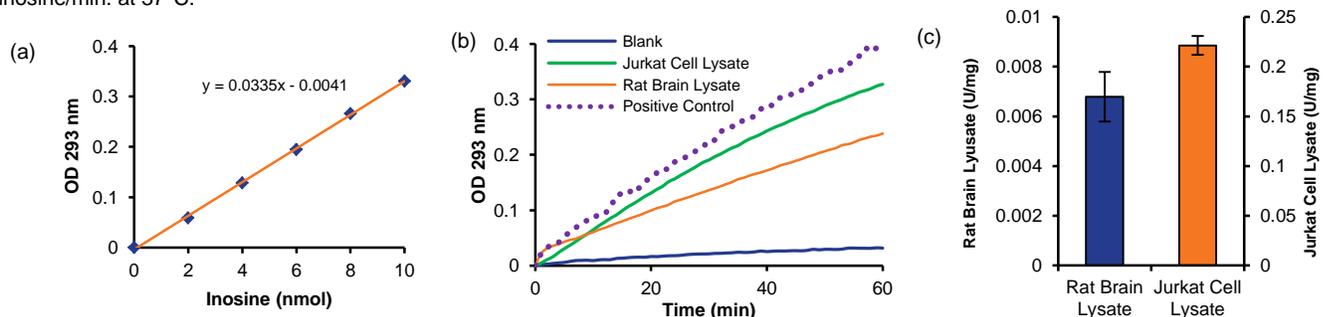


Figure: (a) Inosine Standard Curve, (b) ADA Activity in Jurkat cell (T-lymphocyte) lysate (3 µg), rat brain lysate (19 µg), and Positive Control (c) ADA specific activity in rat brain lysate and Jurkat cell lysate. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

- Xanthine Oxidase Colorimetric/Fluorometric Assay Kit (K710)
- Xanthine/Hypoxanthine Colorimetric/Fluorometric Assay Kit (K685)
- Adenosine Activity Assay Kit (Fluorometric) (K327)
- Ammonia Colorimetric Assay Kit (K370)
- Purine Nucleoside Phosphorylase Activity Assay Kit (Fluorometric) (K767)

- Inosine Fluorometric Assay Kit (K712)
- Uric Acid Colorimetric/Fluorometric Assay Kit (K608)
- Adenosine Deaminase Activity Assay Kit (Fluorometric) (K328)
- Ammonia Colorimetric Assay Kit II (K470)

FOR RESEARCH USE ONLY! Not to be used on humans.