Technical Bulletin

Adenosine Deaminase Activity Assay Kit

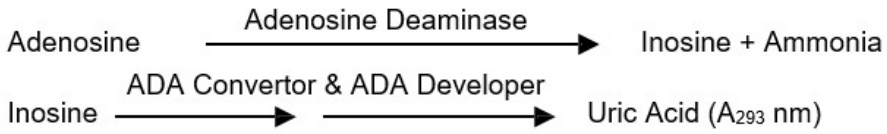
Catalog Number MAK400

Product Description

Adenosine Deaminase (ADA) 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 and nucleus, and it is 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, and chronic active hepatitis. Adenosine Deaminase is also a marker for T-lymphocyte proliferation.

In the Adenosine Deaminase Activity Assay Kit method, inosine formed from the breakdown of adenosine is converted to uric acid by ADA Convertor and ADA Developer. The uric acid is measured at 293 nm. The kit measures total activity of Adenosine Deaminase with lower limit of quantification of 1 mU Adenosine Deaminase.

The kit is suitable for the detection of Adenosine Deaminase (ADA) activity in purified recombinant proteins, nuclear extracts, and cell and tissue lysate.



Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- ADA Assay Buffer (10x) 25 mL
  Catalog Number MAK398A
- ADA Convertor 1 vial
  Catalog Number MAK398B
- ADA Developer 1 vial
  Catalog Number MAK398C
- ADA Substrate 500 µL
  Catalog Number MAK398D
- ADA Positive Control 1 vial
  Catalog Number MAK398E
- Inosine Standard (10 mM) 100 µL
  Catalog Number MAK398F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well clear flat-bottom plate suitable for UV spectral readings. Cell culture or tissue culture treated plates are **not** recommended
- Spectrophotometric multiwell plate reader
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥16,000 × *g*
- Rotary Shaker
- Protease Inhibitor Cocktail (Catalog Number P8340)
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1)

Precautions and Disclaimer

For Research use only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

ADA Assay Buffer (1×): Prepare a 1× buffer by adding one part of ADA Assay Buffer (10x) to nine parts of purified water. Store at -20 °C or 2-8 °C. Bring buffer to 37 °C prior to use. Chill an appropriate amount of 1× ADA Assay Buffer for use in Sample Preparation and supplement with Protease Inhibitor Cocktail.

ADA Converter and ADA Developer: Reconstitute each vial with 210 µL of 1× 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 cycles.

ADA Substrate: Aliquot and store at -20 °C. **Avoid repeated freeze/thaw cycles.**

ADA Positive Control: Reconstitute vial with 25 µL of 1× 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 cycles.

Procedure

Sample Preparation

Tissue

1. Rinse tissue and transfer ~100 mg of fresh or frozen (stored at -80 °C) tissue to a pre-chilled homogenizer.
2. Add 300 µL of cold 1× ADA Assay Buffer containing Protease Inhibitor Cocktail (not included) and thoroughly homogenize tissue on ice.
3. Transfer the tissue homogenate to a cold microfuge tube.
4. Centrifuge the tissue homogenate at 16,000 × *g* at 4 °C for 10 minutes.
5. Transfer the clarified supernatant to a fresh pre-chilled tube and store on ice. Determine the protein concentration using preferred method (BCA is recommended).
6. Use lysates immediately to assay Adenosine Deaminase activity. Alternatively, lysates can be aliquoted and snap frozen in liquid nitrogen before storing at -80 °C. Avoid freeze/thaw cycles.

7. Small molecules such as inosine, xanthine, and hypoxanthine in the samples will contribute to background signal. Remove these molecules by filtering the lysate through a 10kDa Spin Column such as Corning® Spin-X® UF concentrator. Use this modified sample for the assay.
8. Optional: Prepare a parallel Sample well for use as a Sample Background Control (SBC) to ensure that the small molecules are removed by the spin column.
9. Add 2-50 µL of Sample (S) into desired well(s) in 96-well plate. For unknown samples, test different amounts of Sample to ensure the readings are within the Standard Curve range.
10. Adjust the total volume of each Sample (S) well to 50 µL with 1x ADA Assay Buffer.

Cells

1. Add 150-300 µL of cold 1× ADA Assay Buffer containing Protease Inhibitor Cocktail (not included) to 1-5 × 10^6 fresh or frozen cells and pipette several times to disrupt the cells.
2. Transfer cell homogenate including cell debris to a cold microfuge tube and agitate on a rotary shaker at 4 °C for at least 15 minutes.
3. Centrifuge the cell homogenate at 16,000 × *g*, at 4 °C for 10 minutes.
4. Transfer the clarified supernatant to a fresh pre-chilled tube and store on ice. Determine the protein concentration using preferred method (BCA is recommended).
5. Use lysates immediately to assay Adenosine Deaminase activity. Alternatively, lysates can be aliquoted and snap frozen in liquid nitrogen before storing at -80 °C. Avoid freeze/thaw cycles.
6. Small molecules such as inosine, xanthine, and hypoxanthine in the samples will contribute to the background. Remove these molecules by filtering lysate through a 10kDa Spin Column such as Corning® Spin-X® UF concentrator. Use this modified sample for the assay.
7. Optional: Prepare a parallel Sample well for use as a Sample Background Control (SBC) to ensure that the small molecules are removed by the spin column.
8. Add 2-50 µL of Sample (S) into desired well(s) in 96-well plate. For unknown samples, test different amounts of Sample to ensure the readings are within the Standard Curve range.
9. Adjust the total volume of each Sample (S) well to 50 µL with 1× ADA Assay Buffer.

Positive Control

Add 1-2 µL of ADA Positive Control into desired well(s). Adjust the total volume of the well(s) to 50 µL with 1× ADA Assay Buffer.

Sample Background Control (SBC)

Running a Sample Background Control allows for the correction of samples with high background. Use the same amount of tissue/cell lysate as in the Sample (S) well(s). Adjust the total volume to 50 µL/well with 1× ADA Assay Buffer.

Reagent Background Control (RBC)

Add 50 µL of 1× ADA Assay Buffer into desired well(s).

Standard Curve Preparation

Prepare a 1 mM Inosine Standard solution by diluting 10 µL of Inosine Standard (10 mM) with 90 µL of 1× ADA Assay Buffer, mix well. Prepare Inosine Standards according to Table 1. Mix well.

Table 1.
Preparation of Inosine Standards

Well	1 mM Inosine Standard	1x ADA Assay Buffer	Inosine (nmol/ well)
1	0 µL	50 µL	0
2	2 µL	48 µL	2
3	4 µL	46 µL	4
4	6 µL	44 µL	6
5	8 µL	42 µL	8
6	10 µL	40 µL	10

Reaction Mix

1. Mix enough reagents for the number of assays to be performed.
   a. For each well containing Sample (S), Reagent Background Control (RBC), and Positive Control, prepare 50 µL of Reaction Mix according to Table 2. Mix well.
   b. For each well containing Standards and Sample Background Control (SBC), prepare 50 µL of Background Control Mix according to Table 2. Mix well.

Table 2.
Reaction Mix Preparation

Reagent	Reaction Mix	Background Control Mix
1x ADA Assay Buffer	41 µL	46 µL
ADA Convertor	2 µL	2 µL
ADA Developer	2 µL	2 µL
ADA Substrate	5 µL	-

2. Add 50 µL of Reaction Mix into each Sample (S), Reagent Background Control (RBC), and Positive Control well(s).
3. Add 50 µL of Background Control Mix into each Standard and Sample Background Control (SBC) well(s). Mix well.

Measurement

1. Preincubate the plate at 37 °C for 5 minutes.
2. Measure absorbance at 293 nm (A_{293}) in kinetic mode for at least 30 minutes at 37 °C. Read the Inosine Standard Curve along with all test wells.

Results

1. Subtract 0 Standard reading from all Standard Readings.
2. Plot the Inosine Standard Curve.
3. Choose two time points (T_1 & T_2) in the linear range of the plots (with a minimum difference of 2 minutes) and obtain corresponding absorbance (A_{293}) for Sample (S) (i.e., A_{S1} and A_{S2}) and Reagent Background Control (RBC) (i.e., A_{RBC1} and A_{RBC2}).
4. Subtract Reagent Background Control reading from Sample reading to obtain $\Delta A_{S\ Corrected}$:

   $$\Delta A_{S\ Corrected} = (A_{S2}-A_{RBC2}) - (A_{S1}-A_{RBC1})$$

5. Apply the $\Delta A_{S\ Corrected}$ value to the Inosine Standard Curve to determine B nmol of Inosine generated by the Sample during the reaction time ΔT ($\Delta T = T_2 - T_1$).

6. Alternative calculation if the Sample Background Control (SBC) readings are significant.
   a. Substitute the Sample Background Control readings for the Reagent Background Control readings.
   b. Subtract the Sample Background Control (SBC) readings from Sample (S) reading.

   $\Delta A_{S\ Corrected} = (A_{S2}-A_{SBC2}) - (A_{S1}-A_{SBC1})$

   c. Use this $\Delta A_{S\ Corrected}$ value to determine B nmol of Inosine generated by the Sample during the reaction time ΔT ($\Delta T = T_2 - T_1$).
7. Calculate sample ADA activity:

   ADA Activity (nmol/min/μg or mU/μg) =

   $$[B/(\Delta T \times \mu g\ protein)] \times D$$

where:

B = Inosine amount from Standard Curve (nmol).

ΔT = Reaction time ($T_2 - T_1$) (minutes)

μg protein = Amount of protein/well (μg)

D = Sample Dilution factor (if applicable; D = 1 for undiluted Samples)

Sample ADA Activity can also be expressed as U/mg (μmole/minute inosine generated per mg) of protein.

Unit Definition: One unit of Adenosine Deaminase is the amount of enzyme that hydrolyzes adenosine to yield 1.0 μmol of inosine per minute at 37 °C.

Figure 1.
Typical Inosine Standard Curve

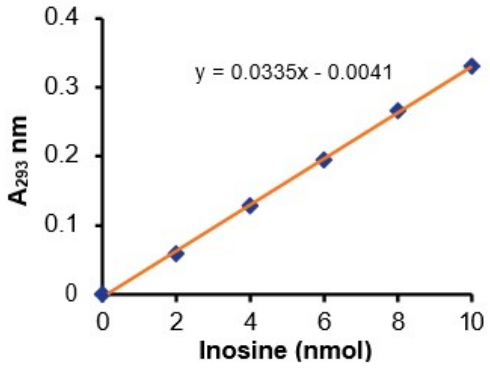


Figure 2.
ADA activity in Jurkat cell (T-lymphocyte) lysate (3 μg), rat brain lysate (19 μg), and Positive Control.

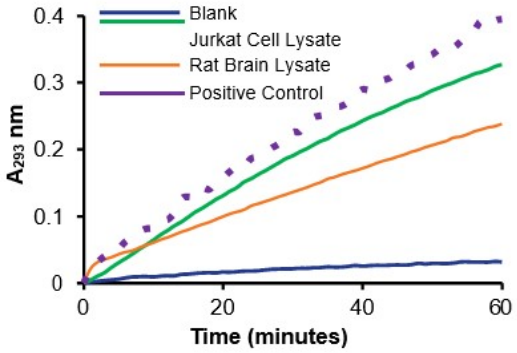
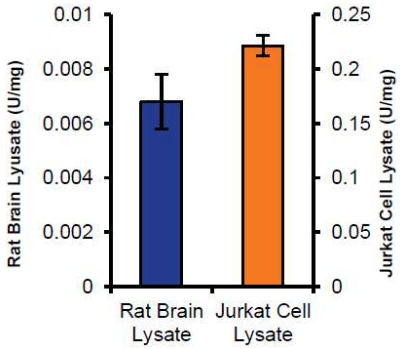


Figure 3.
ADA specific activity in rat brain lysate and Jurkat cell lysate. Assays were performed following the kit protocol.



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