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Product Information

Adenosine Deaminase Activity Assay Kit (Colorimetric)

Catalog Number **EPI023**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product 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 of adenosine deaminase, 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, and chronic active hepatitis to name a few. Adenosine deaminase is also a marker for T-lymphocyte proliferation.

In this 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 of recombinant Adenosine Deaminase.

Components

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

ADA Assay Buffer (10×) Catalog Number EPI023A	25 mL
ADA Convertor Catalog Number EPI023B	1 vial
ADA Developer Catalog Number EPI023C	1 vial
ADA Substrate Catalog Number EPI023D	500 µL
ADA Positive Control Catalog Number EPI023E	1 vial
Inosine Standard (10 mM) Catalog Number EPI023F	100 µL
UV Transparent Plate (96 well) Catalog Number EPI023G	1 plate

Reagents and Equipment Required but Not Provided.

- Spectrophotometric multiwell plate reader
- Protease Inhibitor Cocktail
- Dounce homogenizer

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge small vials at low speed prior to opening. Use ultrapure water for the preparation of reagents.

ADA Assay Buffer (10×) – Make 1× Assay Buffer by adding one volume of 10× Assay Buffer to nine volumes of water. Store at –20 °C or 4 °C. Bring to 37 °C before use.

ADA Convertor and ADA Developer – Reconstitute each with 210 µL of 1× ADA Assay Buffer and mix gently by pipetting. Briefly centrifuge to collect the contents at 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 with 25 µL of 1× ADA Assay Buffer and mix gently by pipetting. Briefly centrifuge to collect the contents at the bottom of the tube. Aliquot and store at –20 °C. Avoid repeated freeze/thaw cycles.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended. Briefly centrifuge all small vials prior to opening.

Procedure
Read entire protocol before performing the assay. All samples and standards should be run in duplicate.

Sample Preparation
Rinse tissue and transfer ~100 mg of fresh or frozen tissue (stored at –70 °C) to a pre-chilled homogenizer. Add 300 µL of cold 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 of cold ADA Assay Buffer containing protease inhibitor cocktail (not provided) to 1–5 × 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 minutes.

Centrifuge the tissue or cell homogenate at 16,000 × *g* at 4 °C for 10 minutes. Transfer the clarified supernatant to a fresh pre-chilled tube and store on ice. Use lysates immediately to assay ADA activity.

Note: Lysates can be aliquoted and snap frozen in liquid nitrogen before storing at –70 °C. Avoid freeze/thaw cycles.

Inosine Standard
Dilute Inosine Standard to 1 mM by adding 10 µL of 10 mM Inosine Standard to 90 µL of 1× 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 nmole/well Inosine Standard. Adjust the volume to 50 µL/well with 1× ADA Assay Buffer.

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 of Positive Control into desired well(s). Make up the volume of samples and Positive Control to 50 µL/well with 1× ADA Assay Buffer. Add 50 µL of 1x ADA Assay Buffer to one well as reagent Background Control.

Notes: For unknown samples, doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range is suggested.

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.

Reaction Mix
Prepare enough reagents for the number of assays to be performed. Make 50 µL of Reaction Mix and Background Control Mix for each well, see Table 1.

Table 1.
Preparation of Mixes

Reagent	Reaction Mix	Background Control Mix
1× 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.

Measurement
Pre-incubate at 37 °C for five minutes and then measure absorbance (OD 293 nm) in kinetic mode for at least thirty minutes at 37 °C. Choose two time points (T_1 and T_2) in linear range (can be as short as 2 minutes) 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.

Results

Calculations

Subtract 0 Standard reading from all Standard Readings. Plot the Inosine Standard Curve. Subtract reagent background control reading from sample reading. Compare the ΔOD $[(A_{S2} – A_{BG2}) – (A_{S1} – A_{BG1})]$ to the Standard Curve to get B (nmole of Inosine) generated by the sample during the reaction time ($\Delta T = T_2 – T_1$).

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 (nmole of Inosine) generated by the sample during the reaction time ($\Delta T = T_2 – T_1$).

$$\text{ADA Activity} = \frac{B}{(\Delta T) \times \mu g \text{ of protein}} \times DF$$

nmol/min/μg = mU/μg

B = Inosine amount from the Standard Curve (nmole)
ΔT = reaction time (minutes)
μg of protein = amount of protein/well (μg)
DF = dilution factor of the sample

Sample ADA Activity can also be expressed as U/mg (μmoles/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 μmole of Inosine/minute at 37 °C.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	Use provided UV Transparent Plate
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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