Version 3b, Last updated 7 November 2023

ab211093 Adenosine Deaminase (ADA) Activity Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement of Adenosine Deaminase (ADA) activity in a variety of samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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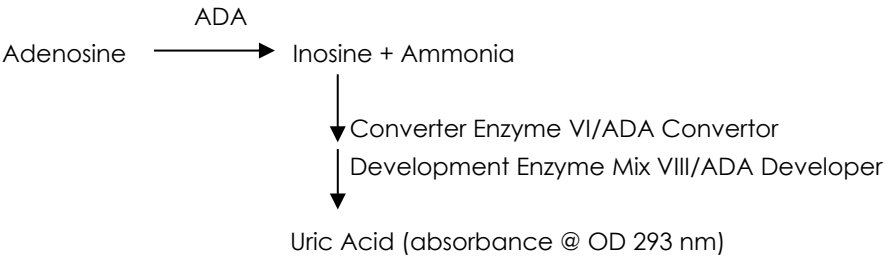
1. Overview

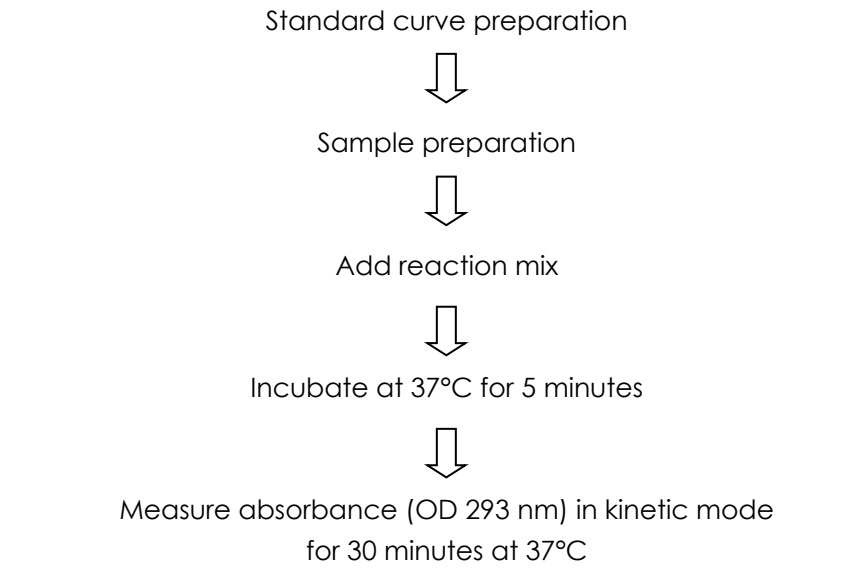
Adenosine Deaminase (ADA) Activity Assay Kit (Colorimetric) (ab204695) is an assay where inosine formed from the breakdown of adenosine is detected via a multi-step reaction, resulting in the formation of an intermediate that reacts with the Converter Enzyme VI/ADA convertor and Development Enzyme Mix VIII/developer to generate uric acid that can be easily quantified at OD293 nm.

The kit measures total activity of Adenosine Deaminase with limit of quantification of 1 mU recombinant Adenosine Deaminase.



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.

2. Protocol Summary



**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
10X ADA Assay Buffer/ADA Assay Buffer (10X)	25 mL	-20°C	4°C / -20°C
Converter Enzyme VI/ADA Convertor (30 U)	1 vial	-20°C	-20°C
Development Enzyme Mix VIII/ADA Developer (10 U)	1 vial	-20°C	-20°C
ADA Positive Control/ADA Positive Control (50 U)	1 vial	-20°C	-20°C
ADA Substrate	500 µL	-20°C	-20°C
Inosine Standard/Inosine Standard (10 mM)	100 µL	-20°C	-20°C
96-Well UV Transparent Plate/UV transparent plate (96-well)	1 unit	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 293 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Cold PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well clear plate with flat bottom
- Dounce homogenizer (if using tissue)
- BCA protein assay kit (reducing agent compatible): we recommend using BCA protein assay kit reducing agent compatible (microplate) (ab207003)
- (Optional) Protease inhibitors: we recommend Protease Inhibitor Cocktail (ab65621) as general use cocktail."

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 10X ADA Assay Buffer/ADA Assay Buffer 10X (25 mL):

Make **1X ADA Assay buffer** by diluting stock 10X ADA Assay Buffer/Assay Buffer (10X) 1:10 in ddH_2O. Diluted buffer can be stored at -20°C or 4°C. Warm to 37°C before use.

9.2 Converter Enzyme VI/ADA Convertor (lyophilized, 30 U):

Reconstitute in 210 µL 1X ADA Assay Buffer and mix gently by pipetting up and down. Aliquot Converter Enzyme VI/convertor so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.3 Development Enzyme Mix VIII/ADA Developer (lyophilized, 10 U):

Reconstitute in 210 µL 1X ADA Assay Buffer and mix gently by pipetting up and down. Aliquot Development Enzyme Mix VIII/developer so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.4 ADA Substrate (500 µL):

Ready to use as supplied. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw.

9.5 ADA Positive Control/ADA Positive Control (lyophilized, 50 U):

Reconstitute in 25 µL 1X ADA Assay Buffer and mix gently by pipetting up and down. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.6 Inosine Standard/Inosine Standard 10 mM (100 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at - 20°C.

9.7 96-Well UV Transparent Plate/UV transparent plate (96-well)

Ready to use as supplied. Equilibrate to room temperature before use. Store at - 20°C.

If you do not use all wells of the plate in one assay, plate can be reused as long as you do not used previous wells and ensure there is no cross-contamination into unused wells.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 1 mM Inosine working standard solution by adding 10 µL of 10 mM Inosine Standard to 90 µL of 1X ADA assay buffer.

10.2 Using 1 mM Inosine working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Inosine 1 mM standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount Inosine in well (nmol/well)
1	0	150	50	0
2	6	144	50	2
3	12	138	50	4
4	18	132	50	6
5	24	126	50	8
6	30	120	50	10

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- If using, add protease inhibitors to sample buffer immediately prior use.

11.1 Cell lysates:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation 1-5 x 10^6 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend cells in 150 – 300 µL cold 1X ADA Assay buffer (containing protease inhibitors).
11.1.4 Homogenize cells quickly by pipetting up and down a few times on ice.
11.1.5 Incubate cell homogenate on a rotary shaker at 4°C for 15 minutes.
11.1.6 Centrifuge sample for 10 minutes at 4°C at 16,000 x*g* using a cold microcentrifuge to remove any insoluble material.
11.1.7 Collect supernatant and transfer to a new pre-chilled tube.
11.1.8 Keep on ice.
11.1.9 Cell lysates may contain small molecules (inosine, xanthine, hypoxanthine) that can interfere with the assay. Remove these from the sample by using a 10 kD Spin Column (ab93349). Add sample to the spin column, centrifuge at 10,000 x*g* for 10 minutes at 4°C. Collect the filtrate.
11.1.10 Measure the amount of protein in the sample.

11.2 Tissue lysates:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation =100 mg).

11.2.2 Homogenize tissue in 300 µL of 1X ADA Assay Buffer (containing protease inhibitors) with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.

11.2.3 Incubate homogenate on ice for 10 minutes.

11.2.4 Centrifuge 10 minutes at 4°C at 16,000 x*g* in a cold microcentrifuge to remove any insoluble material.

11.2.5 Collect supernatant and transfer to a new pre-chilled tube.

11.2.6 Keep on ice.

11.2.7 Tissue lysates may contain small molecules (inosine, xanthine, hypoxanthine) that can interfere with the assay. Remove these from the sample by using a 10 kD Spin Column (ab93349). Add sample to the spin column, centrifuge at 10,000 x*g* for 10 minutes at 4°C. Collect the filtrate.

11.2.8 Measure the amount of protein in the sample.

11.3 Purified protein:

Purified protein can be used directly.

Dilute in ADA Assay Buffer as required.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: Small molecules such as inosine, xanthine and hypoxanthine present in the samples will contribute to the background. Remove them as indicated in the Sample Preparation section. Set up Sample Background Controls to correct for background and to confirm these molecules have been removed.

12.1 Plate Loading:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with ADA Assay Buffer).
- Sample Background control wells = 2 - 50 µL samples (adjust volume to 50 µL/well with ADA Assay Buffer).
- Positive control = 2 µL ADA Positive Control + 48 µL ADA Assay Buffer.
- Reagent Control: 50 µL ADA Assay Buffer.

12.2 ADA Reaction Mix:

12.2.1 Prepare 50 µL of ADA Reaction and Background Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix of the Reaction mix to ensure consistency.

Component	Reaction Mix (µL)	Background Control Mix (µL)
1X ADA Assay Buffer	41	46
Converter Enzyme VI/ADA Convertor	2	2
Development Enzyme Mix	2	2

VIII/ADA Developer		
ADA Substrate	5	0

12.2.2 Add 50 µL of Reaction Mix into each sample, reagent control and positive control wells. Mix well. Do NOT add reaction mix to standard wells or background control wells.

12.2.3 Add 50 µL of Background Control mix to standards and sample background control wells. Mix well.

12.3 Measurement:

12.3.1 Pre-incubate microplate at 37°C 5 minutes.

12.3.2 Measure absorbance at OD = 293 nm on a microplate reader in kinetic mode for at least 30 minutes at 37°C.

Δ Note: Incubation time depends on the ADA activity in the samples. Longer incubation time may be required if activity in the sample is low. We recommend measuring absorbance in a kinetic mode and choosing two time points (T1 and T2) to calculate the ADA activity in the samples. The Inosine Stqndard curve should be read in kinetic mode along with the samples.

13. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

13.1 Standard curve calculation:

13.1.1 Subtract the mean absorbance value of the blank (Standard #1) from all the standard readings. This is the corrected absorbance.

13.1.2 Average the duplicate reading for each standard.

13.1.3 Plot standard curve readings and draw the line of the best fir to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2 Measurement of ADA activity in the sample:

13.2.1 From all reaction wells, choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding absorbance values at those points (A1 and A2).

13.2.2 Calculate ΔOD from sample as follows:

$$\Delta OD = (A2 - ABG2) – (A1 - ABG1)$$

Where BG refers to background reagent or background sample control (see 13.2.3).

13.2.3 If sample background control reading is significant, subtract this value from sample reading instead of reagent background.

13.2.4 ADA activity (nmol/min/µg or mU/µg) in the test samples is calculated as:

$$ADA\ Activity = \left(\frac{B}{\Delta T\ x\ M}\right) x\ D$$

Where:

B = amount of Inosine in the sample well calculated from the standard curve (nmol).

ΔT = reaction time (minutes).

M = amount of protein in the well (µg).

D = sample dilution factor.

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

Unit definition:

1 Unit ADA activity = amount of enzyme that hydrolyzes adenosine to yield 1.0 µmol of Inosine per minute under the assay conditions.

14. Typical Data

Typical standard curve – data provided for **demonstration purposes** only. A new standard curve must be generated for each assay performed.

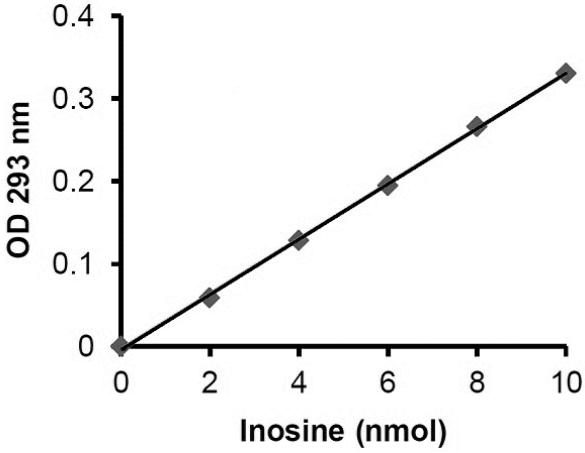


Figure 1. Typical inosine standard calibration curve.

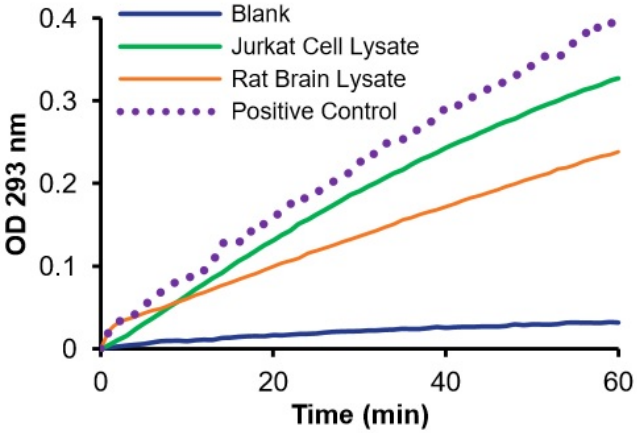


Figure 2. Kinetic curves showing ADA activity detection in positive control (included in the kit), Jurkat cell lysate (3 µg) and rat brain lysate (19 µg).

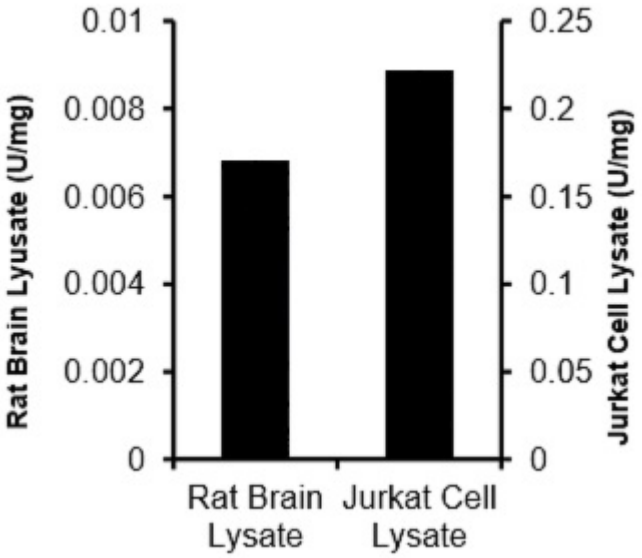


Figure 3. ADA specific activity in rat brain lysate (19 µg) and Jurkat cell lysate (3 µg).

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare Inosine standard, positive control and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare Inosine standard dilution [2 – 10 nmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), sample (50 µL), sample background control (50 µL), reagent background control (50 µL) and positive control wells (50 µL).
- Prepare a master mix for ADA Reaction and Background Mix:

Component	Reaction Mix (µL)	Background Control Mix (µL)
1X ADA Assay Buffer	41	46
Converter Enzyme VI/ADA Convertor	2	2
Development Enzyme Mix VIII/ADA Developer	2	2
ADA Substrate	5	0

- Add 50 µL Reaction mix to sample, reagent background control and positive control wells.
- Add 50 µL of Background Control mix to standards and sample background control wells.
- Pre-incubate microplate at 37°C 5 minutes.
- Measure absorbance at OD = 293 nm on a microplate reader in kinetic mode for at least 30 minutes at 37°C.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ dilute sample so it is within the linear range

17. Interferences

- Small molecules such as inosine, xanthine and hypoxanthine in the samples will contribute to the background. Remove these molecules by using a 10 kD Spin Column (ab93349). Alternatively, pass sample through a desalting column.

18. Notes

Technical Support

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