TECHNICAL MANUAL

Adenosine Deaminase (ADA) Activity Assay Kit

- **SKU CODE:** MAES0141
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and measure absorbance
- Calculate ADA activity

2. Intended use

This kit can be used to detect adenosine deaminase (ADA) activity in serum, plasma, and animal tissue samples.

3. Detection principle

Adenosine deaminase (ADA) hydrolyzes the substrate adenosine to form hypoxanthine riboside, which is subsequently hydrolyzed by purine riboside phosphatase to produce hypoxanthine and ribose phosphate. Under the action of xanthine oxidase, hypoxanthine produces hydrogen peroxide, which reacts with peroxidase, 4-aminoantipyrine, and a color source to produce a red substance. This red substance has a maximum absorption peak at 550 nm, and the changes in absorbance are proportional to ADA activity.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	Working Solution	10 mL x 1 vial	20 mL x 1 vial	50 mL x 2 vials	2-8 °C, 12 months, shading light
Reagent 2	Chromogenic Agent	5 mL x 1 vial	10 mL x 1 vial	50 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 3	1 mmol/L Standard	2 mL x 1 vial	4 mL x 1 vial	4 mL x 5 vials	2-8 °C, 12 months

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Micropipette, Centrifuge, Microplate reader (550 nm), Water bath, Incubator

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Note: The reagents must be stored strictly according to the preservation conditions in the table above. Reagents from different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to ensure sufficient reagent recovery.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 mmol/L standard solution with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0 mmol/L.

7. Sample preparation

Sample preparation:

Serum (plasma): Detect directly. If not detected on the same day, serum or plasma can be stored at -80 °C for one month.

Tissue samples:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4 °C.

4. Centrifuge at 10,000 x g for 10 min to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factors for different samples are as follows (for reference only):

- 10% Mouse liver tissue homogenate: 1
- 10% Rat kidney tissue homogenate: 1
- 10% Rat heart tissue homogenate: 2-3
- 10% Rat spleen tissue homogenate: 2-3
- Mouse serum: 1
- Human serum: 1

Note: The diluent is normal saline (0.9% NaCl). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. It is recommended to use a multichannel pipette when adding chromogenic agent. Add quickly and control the time within 2 minutes.
2. After adding chromogenic agent, incubate at 37 °C for 7 minutes before detection.
3. Prevent bubble formation when transferring reagents or samples into the microplate.
4. There is no change in OD value of standard wells; plot the standard curve using the A2 OD values.

9. Operating steps

1. Standard wells: Add 10 µL of standard with different concentrations into standard wells. Sample wells: Add 10 µL of sample into sample wells.
2. Add 180 µL of working solution to each well.
3. Add 90 µL of chromogenic agent to each well.
4. Incubate at 37 °C for 7 minutes.
5. Measure the OD values of sample wells at 550 nm with microplate reader, recorded as A1.

6. Continue incubation at 37 °C for 10 minutes. Measure the OD values of standard and sample wells at 550 nm with microplate reader, recorded as A2.

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using the absolute OD value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel). (There is no change in OD value of standard wells; plot the standard curve with the A_2 OD values).

The sample:

1. Serum (plasma) sample

Definition: The amount of 1 μmol hypoxanthine riboside produced by 1 L serum (plasma) per minute when catalyzing substrate at 37 °C is defined as 1 activity unit.

ADA activity (U/L) = $(A_2 - A_1 - b) \div a \times 1000^* \div T \times f$

2. Tissue sample:

Definition: The amount of 1 μmol hypoxanthine riboside produced by 1 g tissue protein per minute when catalyzing substrate at 37 °C is defined as 1 activity unit.

ADA activity (U/gprot) = $(A_2 - A_1 - b) \div a \times 1000^* \div T \times f \div C_{pr}$

[Note]

A_1: The OD value after the first incubation for 7 minutes

A_2: The OD value after the second incubation for 10 minutes

T: The second incubation time, 10 minutes

1000*: 1 mmol = 1000 μmol

C_{pr}: Concentration of protein in tissue sample, gprot/L

f: Dilution factor of the sample before testing

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.20	34.50	75.00
%CV	3.4	2.8	2.8

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.20	34.50	75.00
%CV	6.1	5.9	6.0

Recovery

Three samples of high, middle, and low concentrations were tested with 6 parallel replicates for each concentration to obtain an average recovery rate of 98%.

Recovery

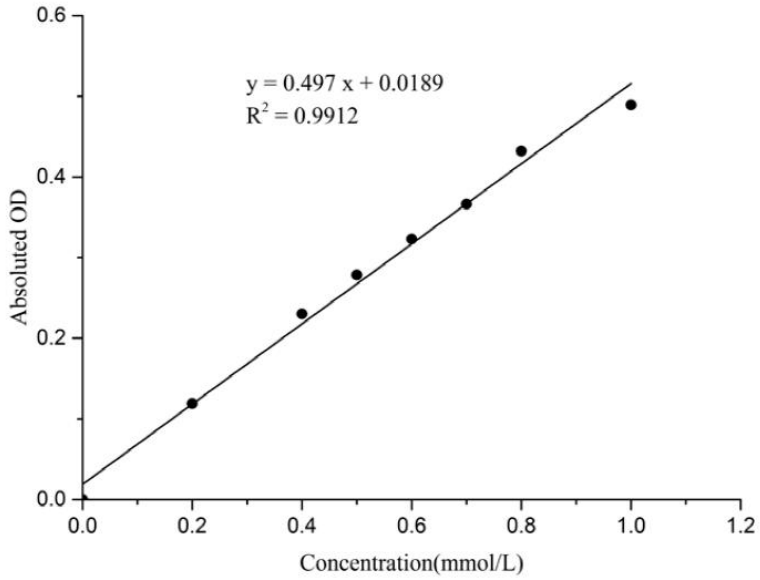
	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.25	0.56	0.74
Observed Conc. (mmol/L)	0.3	0.5	0.7
Recovery rate (%)	103	95	96

Sensitivity

The analytical sensitivity of the assay is 0.03 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve data

As the OD values of the standard curve may vary according to actual assay performance conditions (e.g., operator, pipetting technique, or temperature effects), the standard curve and data provided below are for reference only.



Standard curve data

Concentration (mmol/L)	OD value		Average OD	Absolute OD
0	0.044	0.043	0.044	0.000
0.2	0.162	0.163	0.163	0.119
0.4	0.274	0.273	0.274	0.230
0.5	0.318	0.326	0.322	0.279
0.6	0.361	0.372	0.367	0.323
0.7	0.406	0.414	0.410	0.367
0.8	0.462	0.489	0.476	0.432

Concentration (mmol/L)	OD value		Average OD	Absolute OD
1.0	0.533	0.533	0.533	0.490

12. Appendix II Example Analysis

Example analysis:

For rat liver tissue, take 10 μL of prepared sample and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.497x + 0.0189

A_1 of the sample: 0.177

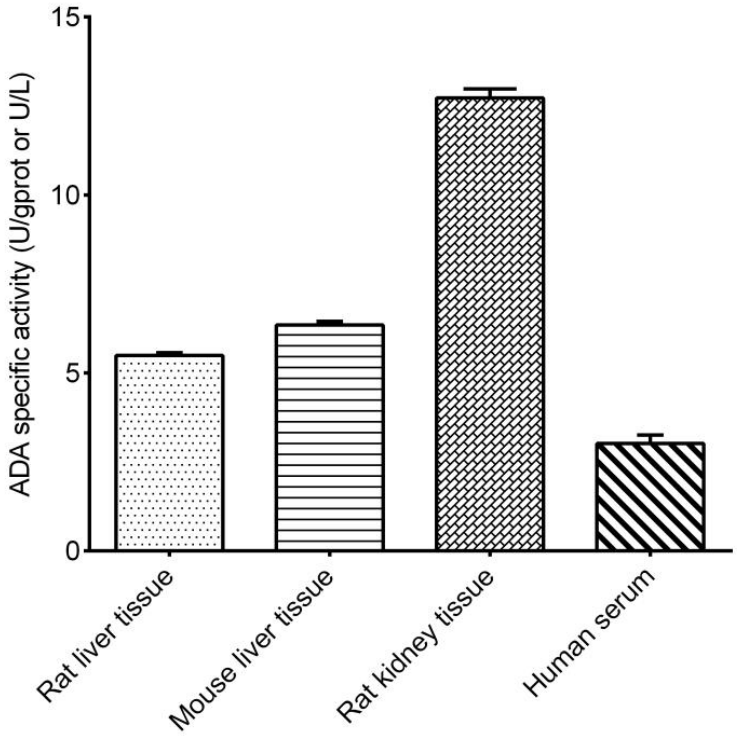
A_2 of the sample: 0.419

Concentration of protein in sample: 8.27 gprot/L

Calculation result:

ADA activity (U/gprot) = (0.419 - 0.177 - 0.0189) ÷ 0.497 ÷ 8.27 ÷ 10 × 1000 = 5.42 U/gprot

Detection of 10% rat liver tissue homogenate (protein concentration: 8.27 gprot/L), 10% mouse liver tissue homogenate (protein concentration: 8.46 gprot/L), 10% rat kidney tissue homogenate (protein concentration: 3.33 gprot/L), and human serum according to the protocol yielded the following results:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pretest if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.