Technical Manual

Adenosine Deaminase (ADA) Activity Assay Kit

- Catalogue Code: MAES0141
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.03-99 U/L

Sensitivity:

0.03 U/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Adenosine deaminase (ADA) is a nucleic acid metabolic enzyme that plays an important role in cellular immune activity. ADA is widely distributed in various tissues of human body, with the highest content in thymus, spleen and other lymphoid tissues, and the lower content in liver, lung, kidney and skeletal muscle. ADA activity is a sensitive index reflecting liver injury, which can be used as one of the routine examination items of liver function. ADA, together with ALT or GGT can comprehensively reflect the enzymatic changes of liver disease.

3. Intended Use

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

4. Detection Principle

Adenosine deaminase (ADA) can hydrolyzed the substrate adenosine to form hypoxanthine riboside, which is hydrolyzed by purine riboside phosphatase to produce hypoxanthine and phosphate ribose. Under the action of xanthine oxidase, hypoxanthine produces hydrogen peroxide, which produces red substance under the action of peroxidase, 4-aminotepyrine and color source. The red substance has the maximum absorption peak at 550 nm and the changes of absorbance is proportional to the activity of ADA.

5. Kit components & storage

Item	Specification	Storage
Working Solution	20 mL × 1 vial	2-8°C, 6 months, away from direct sunlight
Chromogenic Agent	10 mL × 1 vial	2-8°C, 6 months, away from direct sunlight
Standard (1 mmol/L)	2 mL × 2 vials	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Water bath
- Incubator
- Centrifuge
- Microplate Reader (550 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal saline (0.9% NaCl)

6. Assay Notes:

1. It is recommended to use a multichannel pipettor when adding chromogenic agent, add quickly and the time was controlled within 2 min.

2. After adding chromogenic agent, it is necessary to incubate at 37°C for 7 min before detection.

3. Prevent the formulation of bubbles when the reagent or sample is transferred into the microplate.

4. There is no change in OD value of standard well, plot the standard curve with the OD value of A_2.

7. Reagent preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation

1. **Serum sample:**
   Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. **Plasma sample:**
   Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. **Tissue sample:**
   Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of Normal saline (0.9% NaCl) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it

on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.03-99 U/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
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).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 550 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute 1 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0 mmol/L.

The measurement of samples

1. **Standard well:** add 10 µL of standard with different concentrations into standard wells.
   Sample well: 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 min.

5. Measure the OD values of sample wells at 550 nm with microplate reader, recorded as A_1.

6. Continue incubate at 37°C for 10 min. Measure the OD values of standard and sample wells at 550 nm with microplate reader, recorded as A_2.
   Note: Standard wells only need to detect A_2, sample wells need to detect A_1 and A_2.

Operation Table

	Standard well	Sample well
Sample (μL)		10
Standard of different concentrations (μL)	10	
Working Solution (μL)	180	180
Chromogenic Agent (μL)	90	90
Incubate at 37°C for 7 min and measure the OD values of sample wells at 550 nm with microplate reader, recorded as A_1.		
Continue to incubate at 37°C for 10 min. Measure the OD values of standard and sample wells at 550 nm with microplate reader, recorded as A_2.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. ADA activity in Serum (plasma) sample:

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

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

2. Tissue sample:

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

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

y: $\Delta A_{Standard} - \Delta A_{Blank}$ (ΔA_{Blank} is the change OD value when the standard concentration is 0).

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

A_1: The OD value after the first incubation for 7 min;

A_2: The OD value after the second incubation for 10 min;

T: The second incubation time, 10 min;

1000*: 1 mmol =1000 μmol

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

f: dilution factor of the sample before tested.

Email: info@assaygenie.com **Web:** www.assaygenie.com

12. Performance Characteristics

Detection Range	0.03-99 U/L
Sensitivity	0.03 U/L
Average recovery rate (%)	98
Average inter-assay CV (%)	6
Average intra-assay CV (%)	3

Analysis

For rat liver tissue, take 10 μL of prepared sample and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.7395 x + 0.0148, the A1 of the sample is 0.177, the A_2 of the sample is 0.419, the concentration of protein in sample is 8.27 gprot/L, and the calculation result is:

$$\begin{aligned} &\boldsymbol{ADA\ activity\ (U/gprot)} \\ &\quad = (\mathbf{0.419 - 0.177 - 0.0148}) \div \mathbf{0.7395} \div \mathbf{8.27} \div \mathbf{10} \times \mathbf{1000} \\ &\quad = \mathbf{3.71\ U/gprot} \end{aligned}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

Notes:

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