PRODUCT INFORMATION & MANUAL

Adenosine Deaminase/ ADA Activity Assay Kit (Colorimetric)

NBP3-24456

For research use only.
Not for diagnostic or therapeutic procedures.

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Adenosine Deaminase/ADA Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24456

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.03 U/L

Detection range: 0.03-99 U/L

Average intra-assay CV (%): 3.0

Average inter-assay CV (%): 6.0

Average recovery rate (%): 98

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Working Solution	20 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Chromogenic Agent	10 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 3	1 mmol/L Standard	4 mL × 1 vial	2-8℃, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲Materials prepared by users

Instruments

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

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲The key point of the assay

1. It is recommended to use the multichannel pipeter when adding reagent 2, add quickly and the time was controlled within 2 min.
2. After adding reagent 2, 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.

Pre-assay preparation

▲Reagent preparation

Bring all reagents to room temperature before use.

▲Sample preparation

1. Serum (plasma) and urine: Detect the sample directly.
2. Tissue: Weigh the tissue accurately. Add normal saline (0.9% NaCl) in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break tissue fully. Then centrifuge at 10000 g for 10 min at 4℃ and collect the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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).

Assay protocol

▲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.

▲Detailed operating steps

1.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. Reference is as follows:

Number	Standard concentrations (mmol/L)	1mmol/L Standard (μL)	Double distilled water (μL)
A	0	0	200
B	0.2	40	160
C	0.4	80	120
D	0.5	100	100
E	0.6	120	80
F	0.7	140	60
G	0.8	160	40
H	1.0	200	0

2.The measurement of sample

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 reagent 1 to each well.
3) Add 90 μL of reagent 2 to each well.
4) Incubate at 37℃ 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℃ 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.

▲Summary operation table

	Standard well	Sample well
Sample (μL)		10
Standard of different concentrations (μL)	10	
Reagent 1 (μL)	180	180
Reagent 2 (μL)	90	90
Incubate at 37℃ for 7 min and measure the OD values of sample wells at 550 nm, recorded as A_1.		
Continue to incubate at 37℃ for 10 min. Measure the OD values of standard and sample wells at 550 nm, recorded as A_2.		

▲Calculation

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

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℃ is defined as 1 activity unit.

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

2. ADA activity in tissue sample:

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

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

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the 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

Appendix I Data

▲Example 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 A_1 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:

ADA activity (U/gprot)=（0.419-0.177-0.0148）÷0.7395÷8.27÷10×1000
=3.71 U/gprot

Appendix II References

1. Kinoshita H, Yoshida D, Miki K, et al. An amperometric-enzymatic method for assays of inorganic phosphate and adenosine deaminase in serum based on the measurement of uric acid with a dialysis membrane-covered carbon electrode[J]. Analytica Chimica Acta,1995,303: 301-307.
2. Pedersen R C, Berry A J. Sensitive, optimized assay for serum AMP deaminase.[J]. Clinical Chemistry, 1977,9: 1726-1733.