（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K197-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（550 nm）

Detection range：0.03-99 U/L

Elabscience® Adenosine Deaminase (ADA) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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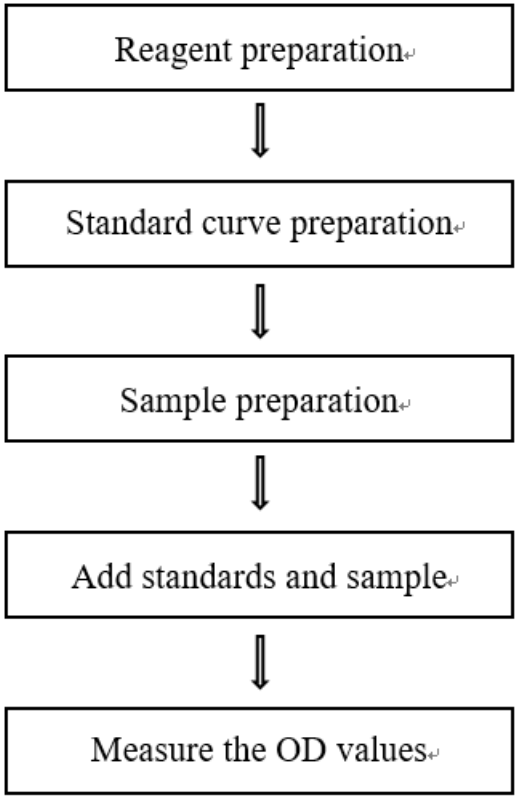
Assay summary



Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Working Solution	10 mL ×1 vial	20 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 2	Chromogenic Agent	5 mL ×1 vial	10 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 3	1 mmol/L Standard	2 mL ×1 vial	4 mL ×1 vial	2-8℃, 12 months
	Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The 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 diluent 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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**1.0**
1 mmol/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation:

Serum (plasma): detect directly. If not detected on the same day, the serum or plasma can be stored at -80 ℃ for a 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℃.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① It is recommended to use the multichannel pipeter 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℃ 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 $A_{2.}$

Operating steps

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 ℃ 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration 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 well, plot the standard curve with the OD value of A_2)

The sample:

1. 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. 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]

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 Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	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

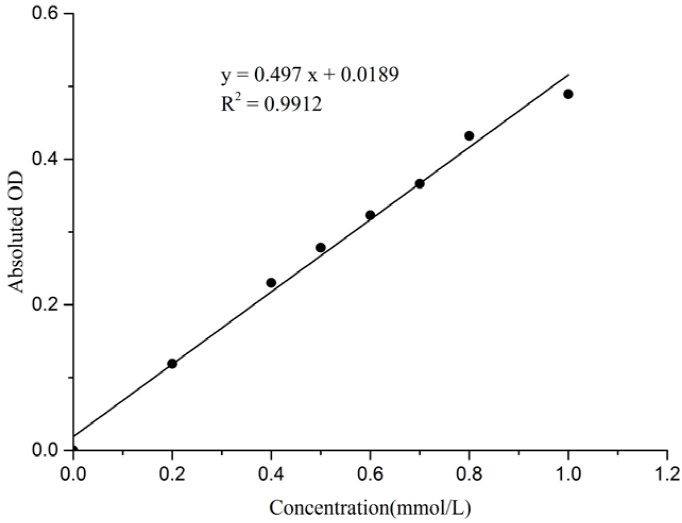
Sensitivity

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.

2. Standard curve

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

Concentration (mmol/L)	**0**	**0.2**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**1.0**
OD value	0.044	0.162	0.274	0.318	0.361	0.406	0.462	0.533
	0.043	0.163	0.273	0.326	0.372	0.414	0.489	0.533
Average OD	0.044	0.163	0.274	0.322	0.367	0.410	0.476	0.533
Absoluted OD	0.000	0.119	0.230	0.279	0.323	0.367	0.432	0.490



Appendix II Example Analysis

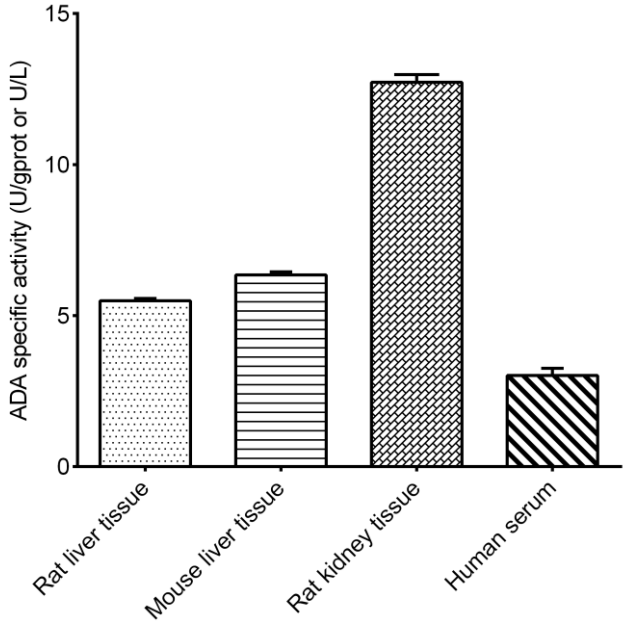
Example analysis:

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

standard curve: y = 0.497 x + 0.0189, 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 - 0.0189) ÷0.497 ÷8.27 ÷10 ×1000 =5.42 U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 8.27 gprot/L), 10% mouse liver tissue homogenate (the concentration of protein is 8.46 gprot/L), 10% rat kidney tissue homogenate (the concentration of protein is 3.33 gprot/L) and human serum according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.