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

Catalog No：E-BC-K909-M

Specification：48T(22 samples)/96T(46 samples)

Measuring instrument：Microplate reader(340nm)

Detection range：1.27-182.52 U/L

Elabscience® Asparaginase (ASNase) Activity Colorimetric 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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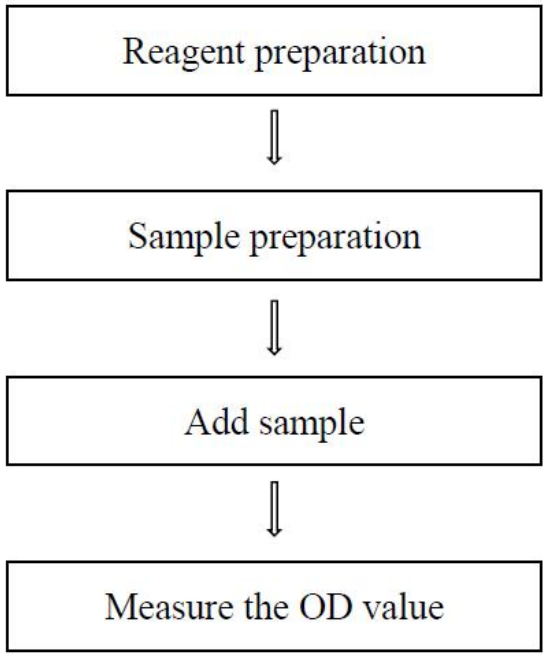
Assay summary



Intended use

This kit can be used to measure asparaginase (ASNase) activity in serum, plasma samples.

Detection principle

Asparaginase (ASNase) is a proteolytic enzyme that was first discovered in guinea pigs and is not naturally produced in humans. Asparaginase has been widely used in the food industry, inhibiting the production of acrylamide carcinogens by hydrolyzing asparagine. Asparaginase is also used to treat various diseases due to its unique anti-tumor properties, such as acute lymphocytic leukemia, non-Hodgkin's lymphoma, leukemia meningitis, lung cancer and ovarian cancer and other tumor diseases.

The detection principle of this kit is as follows: asparaginase catalyzes the substrate to generate aspartic acid, and aspartic acid is hydrolyzed under the catalytic action of the enzyme. The amount of NADH consumed by the reaction is directly proportional to the activity of asparaginase. NADH is absorbed at a wavelength of 340 nm, and the activity of asparaginase can be calculated based on the change in absorbance value per unit time.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	9 mL × 1 vial	18 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Coenzyme	Powder× 1 vial	Powder× 2 vials	-20℃, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder × 1 vial	Powder× 2 vials	-20℃, 12 months, shading light
Reagent 4	Substrate	0.1 mL × 1 vial	0.2 mL × 1 vial	-20℃, 12 months, shading light
	UV-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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (340 nm), Incubator

Reagents:

PBS(0.01 M，pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of coenzyme working solution：

Dissolve one vial of coenzyme with 220 μL of double distilled water, mix well to dissolve. Store at -20°C for a week protected from light.

③ The preparation of enzyme working solution：

Dissolve one vial of enzyme regent with 220 μL of double distilled water, mix well to dissolve. Store at -20°C for a week protected from light.

④ The preparation of working solution：

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 395 μL of working solution (mix well 375 μL of buffer solution, 10 μL of coenzyme working solution and 10 μL of enzyme working solution). The prepared solution should be prepared on spot and used up within 2 h.

⑤ The preparation of substrate working solution：

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 250 μL of substrate working solution (mix well 20 μL of substrate and 230 μL of double distilled water). Store at -20°C for 1 month protected from light.

Sample preparation

① Sample preparation

Serum and plasma: Detect directly.

② Dilution of sample

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

Sample type	Dilution factor
Guinea pig serum	2-15
Guinea pig plasma	2-15

Note: The diluent is PBS(0.01 M，pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Control blank well: Add 40 μL of double distilled water into the wells.

Control well/Sample blank well: Add 20 μL of double distilled water into the wells.

Sample well: Add 20 μL of substrate working solution into the wells.

② Add 20 μL of substrate working solution into sample blank wells.

Add 20 μL of sample into control wells and sample wells.

③ Add 150 μL of working solution into each well.

④ Measure the OD values of each well at 340 nm with microplate reader, as A_1. Incubate at 37℃ for 10 min, measure the OD values of each well at 340 nm with microplate reader, as A_2.

Note: It is recommended to use kinetic detection or record the OD value once every minute for a total of 10 min. Observe whether the OD value change in the sample well within 10 min is a uniform decrease. If it is not a uniform decrease, the sample concentration needs to be adjusted.

Calculation

The sample:

Plasma (serum) samples:

Definition: The amount of NADH in 1 L of plasma (serum) sample that catalyze decomposition of 1 μmol product at 37 ℃ for 1 min is defined as 1 unit.

$$\text{ASNase activity (U/L)} = \frac{(\Delta A_{\text{sample}} - \Delta A_{\text{sample blank}}) - (\Delta A_{\text{control}} - \Delta A_{\text{control blank}})}{\varepsilon \times d} \times \frac{V_1}{V_2} \div T \times f \times 1000$$

[Note]

$\Delta A_{\text{control}}$: The change OD value of control well, $A_1 - A_2$

$\Delta A_{\text{control blank}}$: The change OD value of control blank well, $A_1 - A_2$

ΔA_{sample}: The change OD value of sample well, $A_1 - A_2$

$\Delta A_{\text{sample blank}}$: The change OD value of sample blank well, $A_1 - A_2$

ε: The molar extinction coefficient of at 340 nm, 6.22 L/mmol/cm

d: Optical path, 0.6 cm.

V_1: The volume of total reaction system, 190 μL

V_2: The volume of sample, 20 μL

T: Reaction time, 10 min.

f: Dilution factor of sample before tested

1000：1 mmol = 1000 μmol

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three guinea pig 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)	25.00	50.00	100.00
%CV	2.2	4.7	3.1

Inter-assay Precision

Three guinea pig 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)	25.00	50.00	100.00
%CV	6.7	8.8	8.1

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 98.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	25.00	50.00	100.00
Observed Conc. (U/L)	24.25	50.00	98.00
Recovery rate (%)	97.0	100.0	98.0

Sensitivity

The analytical sensitivity of the assay is 1.27 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.

Appendix II Example Analysis

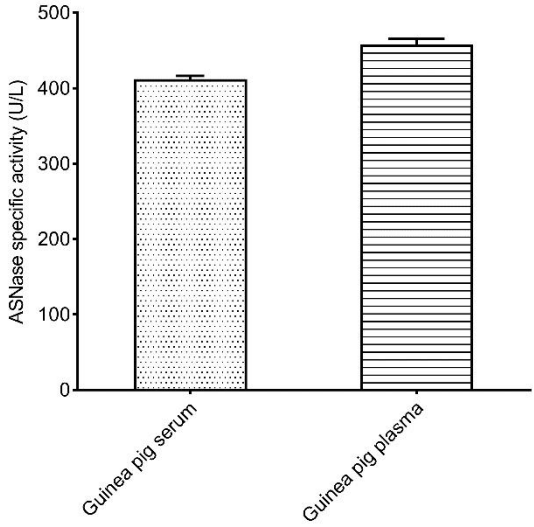
Example analysis：

Take 20 μL of guinea pig plasma (dilute for 6 times) and carry the assay according to the operation table. The results are as follows:

The A_1 value of the sample well is 1.583, the A_2 value of the sample well is 1.262 after the reaction for 10 min, ΔA_{sample} = 1.583 - 1.262 = 0.321. The A_1 value of the sample blank well is 1.627, the A_2 value of the sample blank well is 1.605 after the reaction for 10 min, $\Delta A_{\text{sample blank}}$ = 1.627 - 1.605 = 0.022. The A_1 value of the control well is 1.674, the A_2 value of the control well is 1.649 after the reaction for 10 min, $\Delta A_{\text{control}}$ = 1.674 - 1.649 = 0.025. The A_1 value of the control blank well is 1.622, the A_2 value of the control blank well is 1.600 after the reaction for 10 min, $\Delta A_{\text{control blank}}$ = 1.622 - 1.600 = 0.022, and the calculation result is:

$$\text{ASNase activity (U/L)} = ((0.321 - 0.022) - (0.025 - 0.022)) \div (6.22 \times 0.6) \times (190 \div 20) \div 10 \times 6 \times 1000 = 452.09 \text{ U/L}$$

Detect guinea pig serum (dilute for 3 times), guinea pig plasma (dilute for 6 times), 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.