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

Catalog No：E-BC-K849-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（440-465 nm）

Detection range：1.7-471.7 μmol/L

Elabscience® Aspartate (Asp) 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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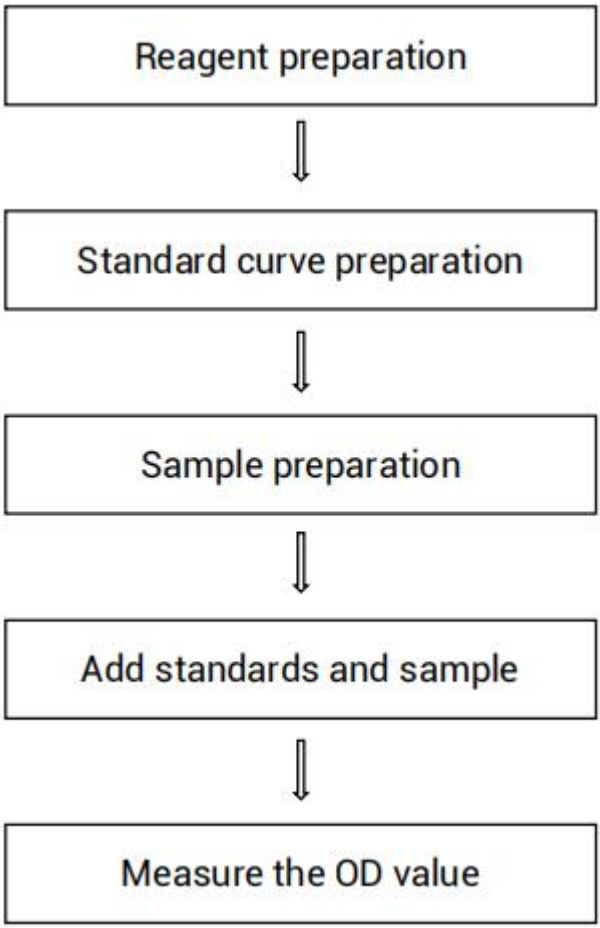
Assay summary



Intended use

The kit can be used to detect the content of aspartate (Asp) in serum, plasma, animal tissue and cell samples.

Detection principle

L-aspartate (L-Aspartate) is an α-amino acid, which is the same acidic amino acid as glutamate. It is one of the non-essential amino acids in the human body. The detection principle of this kit is that the enzyme catalyzes aspartic acid and substrate to produce glutamic acid, and glutamic acid is further catalyzed by the enzyme to produce a product, which will be reduced to orange yellow product under the action of electronic coupler, and the maximum absorption peak is detected at 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	55 mL × 1 vial	-20℃, 12 months
Reagent 2	Substrate	0.5 mL × 1 vial	-20 ℃ , 12 months, shading light
Reagent 3	Catalyst	0.48 mL × 2 vials	-20℃, 12 months, shading light
Reagent 4	Enzymatic Reagent	0.48 mL × 2 vials	-20℃, 12 months, shading light
Reagent 5	Oxidant Reagent	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 6	Chromogenic Agent	5 mL × 1 vial	-20℃, 12 months, shading light
Reagent 7	Accelerant	0.5 mL × 1 vial	-20 ℃ , 12 months, shading light
Reagent 8	500 μmol/L Standard Solution	5 mL × 1 vial	-20 ℃ , 12 months, shading light
	Microplate	96 wells	No requirement

	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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 (440-465 nm, optimum wavelength: 450 nm), Incubator (37℃)

Consumptive material:

3 KD Ultrafiltration tube（Inner tube 1.5 mL，Outer tube 0.5 mL）

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of oxidant working solution：
Dissolve one vial of oxidant reagent with 2.5 mL of double distilled water, mix well to dissolve. Store at -20°C for 3 days protected from light.

③ The preparation of mersuring working solution：
Before testing, please prepare sufficient mersuring working solution according to the test wells. For example, prepare 500 μL of mersuring working solution (mix well 353 μL of buffer solution, 5 μL of substrate, 20 μL of catalyst, 20 μL of enzymatic reagent, 100 μL of oxidant working solution and 2 μL of accelerant). Keep it on ice during use protected from light. The mersuring working solution should be prepared on spot.

④ The preparation of control working solution：

Before testing, please prepare sufficient control working solution according to the test wells. For example, prepare 500 μL of mersuring working solution (mix well 378 μL of buffer solution, 20 μL of enzymatic reagent, 100 μL of oxidant reagent working solution and 2 μL of accelerant). Keep it on ice during use protected from light. The control working solution should be prepared on spot.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 500 μmol/L standard solution with buffer solution diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 150, 200, 300, 350, 400, 500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**150**	**200**	**300**	**350**	**400**	**500**
500 μmol/L standard (μL)	0	40	60	80	120	140	160	200
Buffer solution (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation:

Serum (plasma): Take 100-500 μL samples, filter it through a 3 KD ultrafiltration tube and centrifuge at 12000×g for 10 min. Collect the filtrate for detection.

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 buffer solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and filter it through a 3 KD ultrafiltration tube and centrifuge at 12000×g for 10 min. Collect the filtrate for detection.

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300 μL buffer solution with a ultrasonic cell disruptor at 4℃, centrifuge at 10000×g for 10 min at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and filter it through a 3 KD ultrafiltration tube and centrifuge at 12000×g for 10 min. Collect the filtrate for detection.

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	3-4
10% Mouse heart tissue homogenate	3-4
10% Rat liver tissue homogenate	3-4
10% Rat kidney tissue homogenate	3-4
10% Rat heart tissue homogenate	3-4
Dog serum	1
Horse plasma	1
1.0×10^6 CHO cells	1
1.0×10^6 Jurkat cells	1
1.0×10^6 Molt-4 cells	1
1.0×10^6 293T cells	1
1.0×10^6 HL-60 cells	1
1.0×10^6 THP-1 cells	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into standard wells.
Sample well: add 20 μL of sample into sample wells.
Control well: add 20 μL of sample into control wells.

② Add 200 μL of mersuring working solution to standard wells and sample wells. Add 200 μL of control working solution to control wells.

③ Add 40 μL of chromogenic agent into each well.

④ Mix fully for 5 s with microplate reader, incubate at 37℃ for 40 min with shading light and measure the OD values of each well at 450 nm with microplate reader.

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).

The sample:

1. For serum (plasma) sample

$$\text{Asp content (μmol/L)} = (\Delta A_{450} - b) \div a \times f$$

2. For tissue sample:

$$\text{Asp content (μmol/kg wet weight)} = (\Delta A_{450} - b) \div a \div \frac{m}{V} \times f$$

3. For cell sample:

$$\text{Asp content (μmol/10^6)} = (\Delta A_{450} - b) \div a \div \frac{n}{V} \times f$$

[Note]

ΔA_{450}: $\Delta A_{450} = OD_{sample} - OD_{control}$.

f: Dilution factor of the sample before tested.

m: The wet weight of tissue, g.

n: The number of cell sample,10^6.

V: The volume of homogenate, mL.

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 (μmol/L)	150	290	430
%CV	4.30	2.40	1.90

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 (μmol/L)	150	290	430
%CV	4.40	4.50	9.00

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	150	300	450
Observed Conc. (μmol/L)	151.5	294.0	431.6
Recovery rate (%)	101	98	96

Sensitivity

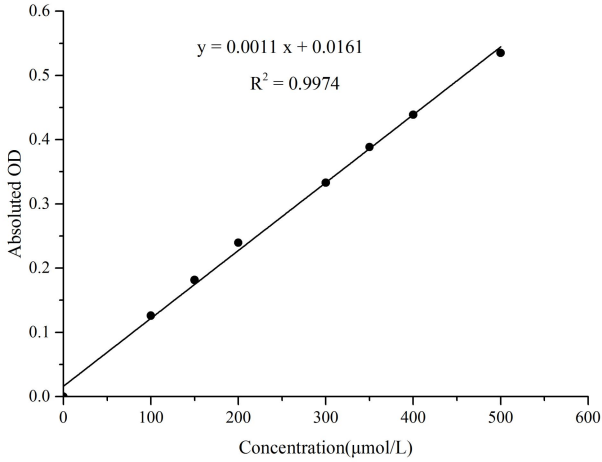
The analytical sensitivity of the assay is 1.7 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding

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 (μmol/L)	0	100	150	200	300	350	400	500
OD value	0.129	0.259	0.320	0.367	0.467	0.523	0.572	0.666
	0.137	0.259	0.309	0.378	0.465	0.520	0.572	0.670
Average OD	0.133	0.259	0.315	0.373	0.466	0.522	0.572	0.668
Absoluted OD	0	0.126	0.182	0.240	0.333	0.389	0.439	0.535



Appendix Π Example Analysis

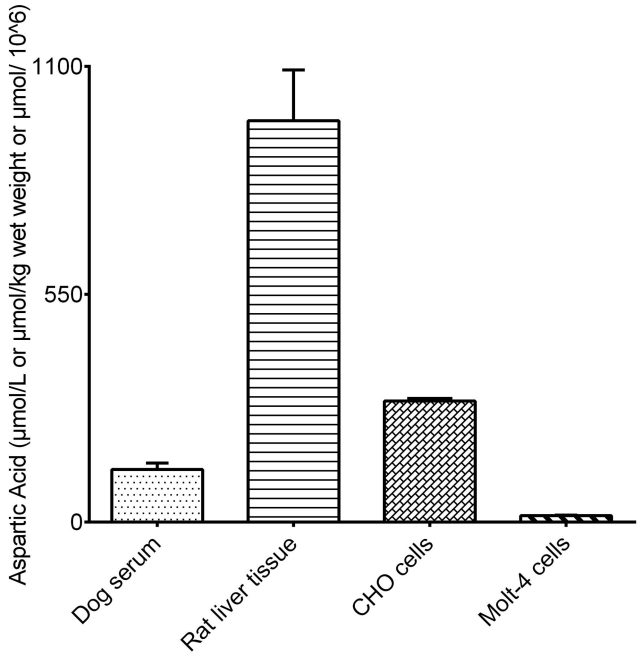
Example analysis：

Take 10% rat liver tissue homogenate, dilute for 3 times, then take 20 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0011 x + 0.0161, The OD value of sample well is 0.510，the OD value of control well is 0.450，$\Delta A_{450} = OD_{sample} - OD_{control}$ = 0.510 - 0.450 = 0.052, the calculation result is:

$$\text{Asp content (μmol/kg wet weight)} = (0.052 - 0.0161) \div 0.0011 \div \frac{0.1}{0.9} \times 3 = 881 \text{ μmol/kg wet weight}$$

Detect dog serum, 10% rat liver tissue homogenate (dilute for 3 times), CHO cells and Molt-4 cells 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.