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

Catalog No：E-BC-K854-M

Specification：48T(44 samples)/96T(92 samples)

Measuring instrument：Microplate reader（330-350 nm）

Detection range：1.95-100 μmol/L

Elabscience®Homocysteine (Hcy) 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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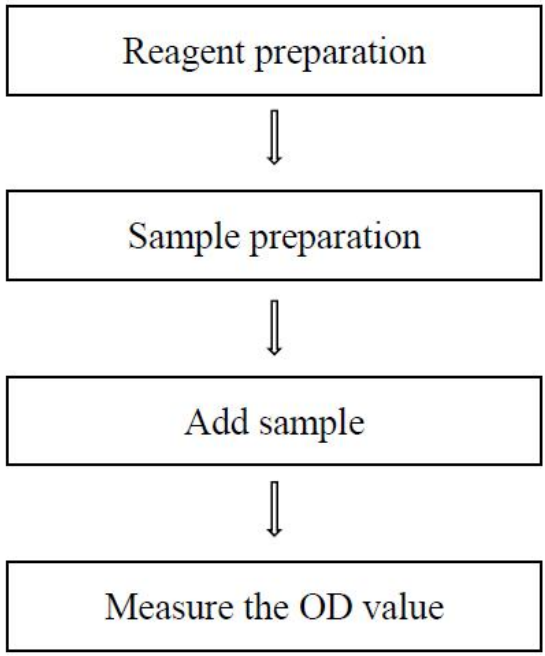
Assay summary



Intended use

This kit can measure homocysteine (Hcy) content in serum (plasma) and urine sample.

Detection principle

Oxidative Hcy is converted into free Hcy, which can convert NADH to NAD^+ through cyclic catalysis of enzymes, resulting in a decrease of OD value at 340 nm. The Hcy content in the sample is calculated by the rate of decrease.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution A	10 mL × 1 vial	20 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Buffer Solution B	6 mL × 1 vial	12 mL × 1 vial	-20℃, 12 months
Reagent 3	Substrate	Power × 1 vial	Power × 2 vials	-20℃, 12 months, shading light
Reagent 4	Enzyme Reagent	Power × 2 vials	Power × 4 vials	-20℃, 12 months, shading light
Reagent 5	28 μmol/L Standard	0.5 mL × 1 vial	1 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 (330-350 nm, optimum wavelength: 340 nm)

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate working solution:
Dissolve one vial of substrate with 1 mL of buffer solution A, mix well to dissolve. Aliquoted storage at -20°C for 5 days protected from light, and avoid repeated freeze/thaw cycles is advised.

③ Preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 200 μL of double distilled water, mix well to dissolve. Store at 2-8°C for 1 day protected from light.

④ Preparation of reaction working solution A:
Before testing, please prepare sufficient reaction working solution A according to the test wells. For example, prepare 160 μL of reaction working solution A (mix well 150 μL of buffer solution A and 10 μL of substrate working solution). Store the reaction working solution A at 2-8°C protected from light. The prepared solution should be used up within 1 day.

⑤ Preparation of reaction working solution B:
Before testing, please prepare sufficient reaction working solution B according to the test wells. For example, prepare 120 μL of reaction working solution B (mix well 110 μL of buffer solution B and 10 μL of enzyme working solution). Store the reaction working solution B at 2-8°C protected from light. The prepared solution should be used up within 1 day.

Sample preparation

① Sample preparation

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

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Human urine	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

The incubation time and determination time should be strictly controlled according to the operation steps.

Operating steps

① Standard well: Add 20 μL of 28 μmol/L Standard to the corresponding wells. Blank well: Add 20 μL of double distilled water to the corresponding wells. Sample well: Add 20 μL of sample to the corresponding wells.

② Add 120 μL of reaction working solution A to each well, then mix fully with microplate reader for 3 s and incubate at 37℃ for 5 min..

③ Add 80 μL of reaction working solution B to each well, then mix fully with microplate reader for 3 s and incubate at 37℃ for 2 min..

④ Measure the OD value of each well at 340 nm recorded as A_1. Incubate at 37℃ for 10 min and measure the OD value of each well at 340 nm recorded as A_2, $\Delta A=A_1 - A_2$.

Calculation

The sample:

Hcy content (μmol/L) = $(\Delta A_{Sample} - \Delta A_{Blank}) \div (\Delta A_{Standard} - \Delta A_{Blank}) \times 28^* \times f$

[Note]

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

$\Delta A_{Standard}$: The change of OD value of standard well, A_1-A_2.

ΔA_{Blank}: The change of OD value of blank well, A_1-A_2.

28*:The concentration of standard, 28 μmol/L.

f: Dilution factor of sample before test.

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)	5.60	35.60	84.30
%CV	2.0	1.7	1.4

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)	5.60	35.60	84.30
%CV	8.8	9.2	9.0

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 104%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （μmol/L）	12.6	42.8	92
Observed Conc. （μmol/L）	13.2	44.1	95.7
recovery rate(%)	105	103	104

Sensitivity

The analytical sensitivity of the assay is 1.95 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.

Appendix II Example Analysis

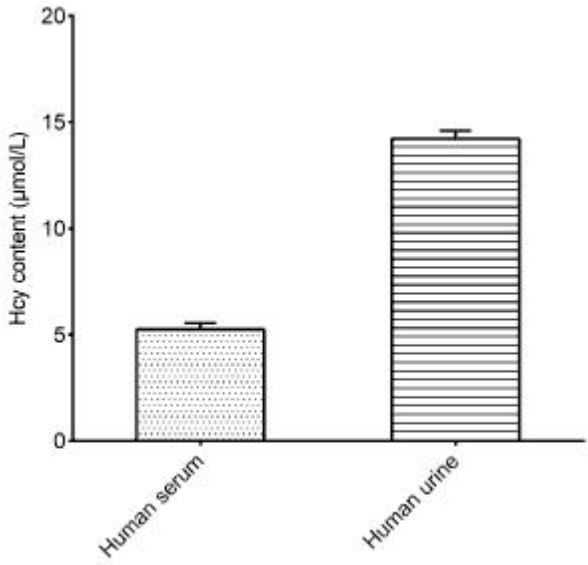
Example analysis:

For human serum, take 20 μL sample and carry the assay according to the operation table. The results are as follows:

tthe A_1 of the sample well is 1.317, the A_2 of the sample well is 1.219, the A_1 of the blank well is 1.107, the A_2 of the blank well is 1.050, the A_1 of the standard well is 1.044, the A_2 of the standard well is 0.795, and the calculation result is:

Hcy content (μmol/L) = (0.098 - 0.057) ÷ (0.249 - 0.057) × 28 = 5.98 μmol/L

Detect human serum , human urine 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.