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

Catalog No：E-BC-K143

Specification：100Assays (Can detect 96 samples with spectrophotometer or 296 samples with biochemical analyzer without duplication)

Measuring instrument：Biochemical analyzer, spectrophotometer

Detection range：0-50 μmol/L

Elabscience® Homocysteine (Hcy) Colorimetric Assay Kit (Enzyme Circulation Method)

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
Tel: 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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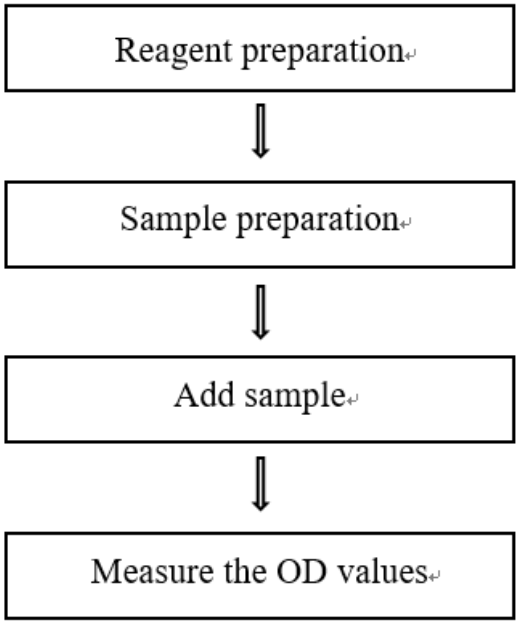
Assay summary



Intended use

The kit is used for the determination of Homocysteine (HCY) in serum samples.

Detection principle

Oxidized homocysteine (HCY) is reduced to free homocysteine by triethyl phosphine (TCEP), and the free homocysteine reacts with substrate to generate adenosine. The generated adenosine is immediately dehydrogenated into inosine and ammonia, and the ammonia is further react with NADH under the catalysis of glutamate dehydrogenase to convert NADH to NAD+. The decrease in absorbance at 340 nm caused by the decline of NADH is proportional to the concentration of homocysteine in the sample.

Kit components & storage

Item	Component	Specification	Storage
Reagent 1	S-adenosylmethionine	37 mL ×2 vials	2-8℃, 12 months, shading light
	NADH		
	Tris (2-carboxyethyl) phosphonium chloride		
	α-ketoglutaric acid		
Reagent 2	HCY methyltransferase	10 mL ×2 vials	2-8℃, 12 months, shading light
	Glutamate dehydrogenase		
	S-adenosine homocysteine hydrolase		
	Adenosine deaminase		
	Mannitol		
	Sodium azide		
Reagent 3	0 μmol/L Homocysteine Standard	1 mL ×1 vial	2-8℃, 12 months
Reagent 4	28 μmol/L Homocysteine Standard	1 mL ×1 vial	2-8℃, 12 months

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:

Biochemical analyzer (340 nm) or Spectrophotometer (340 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

Equilibrate all the reagents to room temperature before use.

Sample preparation

Collect the fasting serum by routine method. The sample is stable at 2-8℃ for 1 week and stable at -20℃ for several months. Do not use serum or plasma containing sodium fluoride. The Sample with hemolysis, turbidity, or severe blood lipid are not suitable for HCY detection. Try to avoid high protein diet before blood collection, which can lead to elevated HCY.

The key points of the assay

① Do not mix reagent 3 and reagent 4. Do not use components from different batches of kit.

② Take the needed amount of reagents and keep the remaining reagent sealed in the refrigerator.

③ The sample needs to be diluted with normal saline before determination once the concentration is beyond the linear range. The result should be multiplied by the dilution factor.

④ Wear rubber gloves when using reagent 2 which contains sodium azide. It should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly and seek for medical treatment if necessary. Other wastes should be treated according to relevant regulations.

Operating steps

1. Detection with Biochemical analyzer

Temperature	37℃	Method	Rate method
Reaction direction	Down	Delay time	120 s
Calibration method	Linear	Detection time	120 s
Sample volume	13 μL	Dominant wavelength	340 nm
Reagent 1	240 μL	Auxiliary wavelength	405 nm
Reagent 2	65 μL		

Automatic biochemical analyzer has its own program parameter input language. Reagents matches the analyzer and carry out automatic measurement after the above basic parameters are modified.

2. Detection with spectrophotometer

	Sample tube	Blank tube	Standard tube
Sample (μL)	39		
Reagent 3 (μL)		39	
Reagent 4 (μL)			39
Reagent 1 (μL)	720	720	720
Mix fully and incubate at 37℃ for 4 min.			
Reagent 2 (μL)	195	195	195
Mix fully and incubate at 37℃ for 2 min. Set the spectrophotometer to zero with distilled water and measure the OD value at 340 nm with a 1 cm optical path cuvette. The OD value of 0 min and 2 min were recorded as A_1 and A_2, respectively. $\triangle A=A_1-A_2$. Calculate $\Delta A/min = (A_1-A_2)/2$ min.			

Calculation

The sample:

$$\text{HCY (μmol/L)} = \frac{\triangle A/\min_{\text{Sample}} - \triangle A/\min_{\text{Blank}}}{\triangle A/\min_{\text{Standard}} - \triangle A/\min_{\text{Blank}}} \times c \times f$$

[Note]

c: Concentration of reagent 4, 28 μmol/L homocysteine standard..

f: Dilution factor of the sample before tested.

Performance index

① A340 of blank ≥ 1.000 (340 nm, 1 cm optical path).

② ΔA/min of blank ≤ 0.0300 (340 nm, 1 cm optical path).

③ Sensitivity: The rate of change in absorbance (ΔA/min) is more than 0.0100 when testing 10 μmol/L samples.

④ Linear range: 0-50 μmol/L, r2 ≥ 0.990.

⑤ The intra-assay CV ≤ 8 %, the inter-assay CV≤ 10 %.

⑥ The relative deviation is 15%~15%.

⑦ Stability: This kit can be store at 2-8℃ with shading light for 12 months. It can be stable for a month at 2-8℃ with shading light after opening.

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.