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

Catalog No: E-BC-K143

Method: Colorimetric method

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

Instrument: Biochemical analyzer, spectrophotometer

Detection range: 0-50 μmol/L

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

General information

Intended use

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

Background

The kit is used for auxiliary diagnosis of related diseases by determining the serum homocysteine concentration. Homocysteine is mainly used as a risk indicator of cardiovascular disease, especially coronary atherosclerosis and myocardial infarction. The increase in homocysteine concentration is proportional to the risk of disease and is an independent risk factor to induce cardiovascular disease.

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℃ , shading light
	NADH		
	Tris (2-carboxyethyl) phosphonium chloride		
	α-ketoglutaric acid		
Reagent 2	HCY methyltransferase	10 mL × 2 vials	2-8℃ , 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℃
Reagent 4	28 μmol/L Homocysteine Standard	1 mL × 1 vial	2-8℃
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.			

▲Materials prepared by users

Instruments

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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

Pre-assay preparation

▲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.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0-50 μmol/L).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	340 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operation table

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 A1 and A2, respectively. ΔA=A1-A2. Calculate ΔA/min = (A1-A2)/2 min.			

▲Calculation

$$HCY(\mu mol/L) = \frac{\Delta A/min_{Sample} - \Delta A/min_{Blank}}{\Delta A/min_{Standard} - \Delta A/min_{Blank}} \times c \times f$$

Note:

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

f: Dilution factor of sample before test.

▲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, $r^2 \geq 0.990$.
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.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.
5. Do not mix reagent 3 and reagent 4. Do not use components from different batches of kit.
6. Take the needed amount of reagents and keep the remaining reagent sealed in the refrigerator.
7. 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.
8. 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.