Technical Manual

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

- Catalogue Code: MAES0115
- Size: 100 Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0-50 µmol/L

Storage:

2-8°C for 12 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 12 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Working solution 1	37 mL × 2 vials	2-8°C, 12 months, avoid direct sunlight
Working solution 2	10 mL × 2 vials	2-8°C, 12 months, avoid direct sunlight
Homocysteine Standard (0 μmol/L)	1 mL × 1 vial	2-8°C, 12 months,
Homocysteine Standard (28 μmol/L)	1 mL × 1 vial	2-8°C, 12 months,

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Biochemical analyzer (340 nm) or Spectrophotometer (340 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Sample Preparation

Collect the fasting serum by routine method. The sample is stable at 2-8°C for 1 week and stable at -20°C 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 prevent high protein diet before blood collection, which can lead to elevated HCY.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample with normal saline according to the result of the pre-experiment and the detection range (0-50 μmol/L).

7. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 340 nm

8. Operation Steps

Operation Table (Detection with Biochemical analyser)

Temperature	37°C	**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
Working solution 1	240 μL	**Auxiliary wavelength**	405 nm
Working solution 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.

Operation Table (Detection with spectrophotometer)

	Sample tube	Blank tube	Standard tube
Sample (μL)	39		
Homocysteine Standard (μL) (0 μmol/L)		39	
Homocysteine Standard (μL) (28 μmol/L)			39
Working solution 1 (μL)	720	720	720
Mix fully and incubate at 37°C for 4 min.			
Working solution 2 (μL)	195	195	195
Mix fully and incubate at 37°C 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.			

9. Calculations

$$\text{Concentration of HCY}\left(\frac{\mu\text{mol}}{\text{L}}\right) = \frac{\triangle\,\text{A/min}_{\text{Sample}} - \triangle\,\text{A/min}_{\text{Blank}}}{\triangle\,\text{A/min}_{\text{Standard}} - \triangle\,\text{A/min}_{\text{Blank}}} \times \text{CStandard}\ \ (28\,\mu mol/L)$$

ΔA/min: rate of change in absorbance per minute

$C_{standard}$: concentration of homocysteine standard , 28 μmol/L

10. Performance Characteristics

Detection Range	0-50 μmol/L

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

Notes:

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