# PRODUCT INFORMATION & MANUAL

# Homocysteine Assay Kit (Colorimetric)

## *NBP3-25830*

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

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

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

**Catalog No:** E-BC-K143
**Method:** Colorimetric method
**Specification:** 100 Assays **(**Can detect 96 samples with spectrophotometer or 296 samples with biochemical analyzer without duplication)
**Measuring instrument:** Biochemistry analyzer, Spectrophotometer
**Detection range:** 0-50 μmol/L

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

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

## Application

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

## Detection significance

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 composition

| | Specification | Component | Concentration |
|---|---|---|---|
| Reagent 1 (R1) | 37 mL ×2 vial | S-adenosylmethionine | 0.1 mmol/L |
| | | NADH | 0.3 mmol/L |
| | | Tris (2-carboxyethyl) phosphonium chloride | 0.5 mmol/L |
| | | α-ketoglutaric acid | 5.2 mmol/L |
| Reagent 2 (R2) | 10 mL ×2 vial | HCY methyltransferase | 6.0 kU/L |
| | | Glutamate dehydrogenase | 12.0 kU/L |
| | | S-adenosine homocysteine hydrolase | 3.8 kU/L |
| | | Adenosine deaminase | 5.6 kU/L |
| | | Mannitol | 0.2% |
| | | Sodium azide | 0.3‰ |
| Standard I | 1 mL ×1 vial | Homocysteine | 0 μmol/L |
| Standard II | 1 mL ×1 vial | Homocysteine | 28.0 μmol/L |

## Applicable instruments

Automatic biochemical analyzer or Spectrophotometer, incubator

## Sample requirements

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 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.

## Operation steps

### 1. Detection with Biochemical analyzer

**Setting parameter**

| 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 I (R1) | 240 μL | Auxiliary wavelength | 405 nm |
| Reagent Ⅱ (R2) | 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

**Operation table**

| | Sample tube | Blank tube | Standard tube |
|---|---|---|---|
| Sample (μL) | 39 | | |
| Standard I (μL) | | 39 | |
| Standard II (μL) | | | 39 |
| Reagent 1 (R1) (μL) | 720 | 720 | 720 |
| Mix fully and incubate at 37℃ for 4 min. | | | |
| Reagent 2 (R2) (μL) | 195 | 195 | 195 |
| Mix fully and incubate at 37℃ for 2 min. Set 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 of results

$$\text{Concentration of HCY (μmol/L)} = \frac{\triangle A/\min_{\text{Sample}} - \triangle A/\min_{\text{Blank}}}{\triangle A/\min_{\text{Standard}} - \triangle A/\min_{\text{Blank}}} \times C_{\text{Standard}}\ (28\ \mu mol/L)$$

ΔA/min: rate of change in absorbance per minute.

$C_{standard}$: concentration of HCY in the Standard II, 28 μmol/L.

## Reference range

0-15 μmol/L (This is for reference only.)

## Performance parameters

1. $A_{340}$ 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. Do not use components from different batches of kit.
4. Do not mix Standard I and Standard II. Do not use components from different batches of kit.
5. Take the needed amount of reagents and keep the remaining reagent sealed in the refrigerator.
6. 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.
7. Wear rubber gloves when using reagent Ⅱ 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.