PRODUCT INFORMATION & MANUAL

Homocysteine Assay Kit (Colorimetric)

NBP3-25825

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

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Homocysteine (Hcy) Colorimetric Assay Kit

Catalog No: NBP3-25825

Method: Colorimetric method

Specification: 96T (Can detect 92 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 1.95 μmol/L

Detection range: 1.95-100 μmol/L

Average intra-assay CV (%): 1.7

Average inter-assay CV (%): 9.0

Average recovery rate (%): 104

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲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	Specification	Storage
Reagent 1	Buffer Solution A	20 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 2	Buffer Solution B	12 mL × 1 vial	-20℃ , 12 months
Reagent 3	Substrate	Power × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Enzyme Reagent	Power × 4 vials	-20℃ , 12 months, shading light
Reagent 5	28 μmol/L Standard	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.			

▲Materials prepared by users

Instruments

Microplate reader (330-350 nm, optimum wavelength: 340 nm).

▲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.

▲The key points of the assay

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

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 3 working solution:
   Dissolve a vial of reagent 3 with 1 mL reagent 1. The prepared solution can be divided into smaller packages at -20℃ with shading light for 5 days and avoid repeated freezing and thawing.
3. Preparation of reagent 4 working solution:
   Dissolve a vial of reagent 4 with 200 μL double distilled water. The prepared solution can be stored at 2-8℃ with shading light for 1 day.
4. Preparation of reaction working solution A:
   Mix reagent 1 and reagent 3 at a ratio of 15:1. The prepared solution should be stored at 2-8℃ with shading light and used up within 1 day.
5. Preparation of reaction working solution B:
   Mix reagent 2 and reagent 4 working solution at a ratio of 11:1. The prepared solution should be stored at 2-8℃ with shading light and used up within 1 day.

▲Sample preparation

Serum (plasma) and urine samples:

Detect the sample directly. If the sample is turbidity, centrifuge at 10000 g for 10 min, then take the supernatant for detection.

▲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 (1.95-100 μmol/L).

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).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	B	B	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	S1	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	S2	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S3	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S4	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S5	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

[Note]：A, blank wells; B, standard wells; S1 - S92, sample wells.

▲Detailed operation steps

1) Standard well: Add 20 μL of reagent 5 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, $\Delta A=A_2-A_1$.

▲Summary operation table

	Standard well	Blank well	Sample well
Reagent 5 (μL)	20		
Double distilled water (μL)		20	
Sample (μL)			20
Reaction working solution A (μL)	120	120	120
Mix fully with microplate reader for 3 s and incubate at 37℃ for 5 min.			
Reaction working solution B (μL)	80	80	80
Mix fully and incubate at 37℃ for 2 min. Measure the OD value of each well recorded as A_1, Incubate at 37℃ for 10 min and measure the OD value recorded as A_2, $\Delta A=A_2-A_1$.			

▲Calculation

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

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 Data

▲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