TECHNICAL MANUAL

Homocysteine (Hcy) Colorimetric Assay Kit

- **SKU CODE:** MAES0267
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add standards and samples
- Add reaction working solution A
- Add reaction working solution B
- Measure absorbance at 340 nm

2. Intended use

This kit can measure homocysteine (Hcy) content in serum (plasma) and urine samples.

3. Detection principle

Oxidized 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.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution A	10 mL x 1 vial	20 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 2	Buffer Solution B	6 mL x 1 vial	12 mL x 1 vial	-20 °C, 12 months
Reagent 3	Substrate	Power x 1 vial	Power x 2 vials	-20 °C, 12 months, shading light
Reagent 4	Enzyme Reagent	Power x 2 vials	Power x 4 vials	-20 °C, 12 months, shading light
Reagent 5	28 μmol/L Standard	0.5 mL x 1 vial	1 mL x 1 vial	-20 °C, 12 months, shading light
	UV Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 1 mL of buffer solution A, mix well to dissolve. Store aliquots at -20 °C for 5 days protected from light, and avoid repeated freeze/thaw cycles.
3. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 200 µL of double distilled water, mix well to dissolve. Store at 2-8 °C for 1 day protected from light.
4. **Preparation of reaction working solution A:** Before testing, prepare sufficient reaction working solution A according to the test wells. For example, prepare 160 µL of reaction working solution A (mix well 150 µL of buffer solution A and 10 µL of substrate working solution). Store the reaction working solution A at 2-8 °C protected from light. The prepared solution should be used within 1 day.
5. **Preparation of reaction working solution B:** Before testing, prepare sufficient reaction working solution B according to the test wells. For example, prepare 120 µL of reaction working solution B (mix well 110 µL of buffer solution B and 10 µL of enzyme working solution). Store the reaction working solution B at 2-8 °C protected from light. The prepared solution should be used within 1 day.

7. Sample preparation

Sample preparation:

Serum (plasma) and urine: detect directly. If not detected on the same day, the sample can be stored at -80 °C for a month.

Dilution of sample:

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). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

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

9. Operating steps

1. Standard well: Add 20 μL of 28 μmol/L Standard 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 °C 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 °C for 2 min.
4. Measure the OD value of each well at 340 nm recorded as A1. Incubate at 37 °C for 10 min and measure the OD value of each well at 340 nm recorded as A2, ΔA=A1 - A2.

10. Calculation

The sample:

Hcy content (μmol/L) = (ΔA_Sample - ΔA_Blank) ÷ (ΔA_Standard - ΔA_Blank) × 28* × f

[Note]

ΔA_Sample: The change of OD value of sample well, A_1-A_2.

ΔA_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.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	5.60	35.60	84.30
%CV	2.0	1.7	1.4

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	5.60	35.60	84.30
%CV	8.8	9.2	9.0

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times in parallel to get the average recovery rate of 104%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	12.6	42.8	92
Observed Conc. (μmol/L)	13.2	44.1	95.7
recovery rate(%)	105	103	104

Sensitivity

The analytical sensitivity of the assay is 1.95 µmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

12. Appendix II Example Analysis

Example analysis:

For human serum, take 20 µL sample and carry the assay according to the operation table. The results are as follows:

The 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

Detect human serum, human urine according to the protocol, the result is as follows:

13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.