HOMOCYSTEINE TEST KIT

Enzymatic Cycling Method (Kinetic UV Decrease)

Product Codes: 12044, 13044, 14044	**Reaction Type:** Enzymatic Rate / UV Kinetic (Decreasing)
Pack Sizes: 2x20 ml, 2x50 ml, 10 ml	**Matrix Target:** Human Serum & Plasma
Storage Temp: 2–8°C (Do Not Freeze)	**Wavelength:** 340 nm (Flow Cell: 37°C)

INTENDED USE & CLINICAL SIGNIFICANCE

Intended Use: This diagnostic reagent system is intended for the quantitative in vitro determination of total Homocysteine (tHcy) levels across human serum or plasma matrices using automated or semi-automated chemistry platforms.

Clinical Significance: Homocysteine (Hcy) is a thiol-containing intermediate sulfur amino acid produced via the intracellular demethylation of methionine. Total homocysteine (tHcy) in circulation represents the sum of oxidized, protein-bound, and free forms. An elevated blood concentration of tHcy (hyperhomocysteinemia) has emerged as an important standalone risk factor for cardiovascular disease, coronary artery anomalies, deep vein thrombosis, and stroke by inducing direct oxidative injury to arterial vessels.

METHOD PRINCIPLE

This method utilizes an advanced enzymatic cycling system. Oxidized homocysteine is reduced to free Hcy, which then reacts with S-adenosylmethionine (SAM) via a recombinant Hcy S-adenosyltransferase to generate S-adenosylhomocysteine (SAH). The secondary co-substrate product releases adenosine via SAH hydrolase. This free adenosine is processed by adenosine deaminase to inosine, driving a kinetic coupled enzyme loop with glutamate dehydrogenase where NADH is oxidized to NAD^+:

$$\text{Homocysteine + SAM } \xrightarrow{[\text{Hcy Transferase}]} \text{ SAH } \rightarrow [\text{SAH Hydrolase}] \rightarrow \text{ Adenosine + Homocysteine (Enzymatic Cycle)}$$

$$\text{Adenosine } \rightarrow [\text{Deaminase}] \rightarrow \text{ Inosine + } NH_3$$

$$NH_3 + \alpha\text{-Ketoglutarate + NADH + } H^+ \xrightarrow{[\text{GLDH}]} \text{L-Glutamate + } NAD^+ + H_2O$$

The rate of decrease in optical absorbance at 340 nm due to NADH oxidation is directly proportional to the total homocysteine concentration in the sample.

STEP 1: REAGENT CONFIGURATION & PIPETTING BASELINE

Reagent / Component Line	Test System Line Volume
R1 - Enzyme / Reduction Reagent Mixture	350 µl
Patient Sample / Calibrator Vector	15 µl
Mix well, incubate for 5 minutes at 37°C to clear baseline tracks. Then add:	
R2 - Cycling Substrate / Enzyme Starter	50 µl

Operational Directive: Mix thoroughly. After an initial 60-second delay post-addition of R2, record the rate of decrease in absorbance at 340 nm per minute (ΔA/min) over a 2-minute monitoring window at 37°C.

STEP 2: CALCULATIONS & DATA TRACKING

tHcy Level (µmol/L) = ***[ΔA/min of Sample / ΔA/min of Calibrator] × Calibrator Concentration***

TECHNICAL PARAMETERS & DIAGNOSTIC SUPPORT LIMITS

Expected Reference	**Healthy Baseline Range:** 5.0 to 15.0 µmol/L. Values above 15 µmol/L define hyperhomocysteinemia. Each individual laboratory must validate and implement its own native local reference parameters.
Linearity Range Limit	Dependably linear and valid up to a threshold of **50.0 µmol/L.**
Over-Limit Protocol	For values reading above 50 µmol/L, pre-dilute the specimen 1:5 or 1:10 with normal saline solution, re-run the assay, and multiply the final arithmetic outcome by the chosen dilution factor.
Safety & Controls	Professional in vitro diagnostic use. Avoid inhalation and direct mucosal contact with reagents. Process normal and abnormal assayed control samples with each analytical batch to validate curve verification.

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