Homocysteine - Liquid UV reagent Ref.:HCY-010
Quantitative Determination of Homocysteine
Only for *in vitro* use in clinical laboratory
Store at 2-8ºC

Homocysteine

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CLINICAL SIGNIFICANCE

Homocysteine (Hcy) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Total homocysteine (tHcy) represents the sum of oxidised, protein bound and free forms of Hcy.
Elevated levels of tHcy have emerged as an important risk factor in the assessment of cardiovascular disease[1-3]. Excess Hcy in the blood stream may cause injury to arterial vessels due to its irritant nature, and result in inflammation and plaque formation, which may eventually cause blockage of blood flow to the heart. Elevated levels of tHcy are also linked with Alzheimers disease[4] and osteoporosis[5].

PRINCIPLE OF THE TEST:

Oxidised homocysteine is reduced to free homocysteine. Free Hcy is converted to cystathionine by the use of CBS (cystathionine beta-synthase) and excess serine. The cystathionine is then broken down to homocysteine, pyruvate and ammonia. Pyruvate is converted to lactate via lactate dehydrogenase with NADH as coenzyme. The rate of NADH conversion to NAD^+ (ΔA_{340nm}) is directly proportional to the concentration of homocysteine.

WARNINGS AND PRECAUTIONS:

- When handling samples, take great care to prevent infection by HBV, HIV, and HCV, wear rubber gloves.
- Be sure not to allow foreign substances including dust, fungi, bacteria, and detergent to get mixed into samples. In addition, take care to prevent contamination of reagents and cuvettes.
- On testing, wear disposable gloves and avoid oral pipetting to prevent infection.
- Dispose of the reagent under a large amount of running water, as each component reagent contains sodium azide. If they get into the eyes or mouth or adhere to skin, first aid measures such as thorough flushing with water should be taken. Consult a physician if necessary.

INSTRUMENTS:

Instrument applications are available upon request.

COMPONENT COMPOSITION:

Component	**Ingredients**	*Concentration in Tests*
Reagent 1	Tris Buffer	--
	LDH	35KU/L
	L-Serine	0.76 mmol/L
	TCEP	0.5 mmol/L
	NADH	0.47 mmol/L
	PRESERVATIVES	---
Reagent 2	Tris Buffer	--
	Cystathionine β-Synthase	20KU/L
	Cystathionine β-lyase	10KU/L
	L-Serinelyase	20KU/L
	PRESERVATIVES	--

REAGENT PREPARATION AND STABILITY:

Reagent is ready for use.

If stored at 2-8℃ and handled properly, component is stable until expiry date stated on the label.

TYPE OF SPECIMEN:

Use serum or heparin plasma as specimen.

It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification.

- Serum/plasma should be separated from cells within 8 hours after collection.
  Stability: 2 weeks at 2-8°C.

TEST PROCEDURE:

Materials required but not supplied:
HCY Controls and Calibrator
General Laboratory Equipment

Assay procedure:

Wavelength: 340 nm
Temperature: 30°C or 37°C
Optical path: 1 cm light path.

STANDARD PROCEDURE:	**Blank**	**Calibrator**	**Sample**
Reagent 1	960 µl	960 µl	960 µl
Sample	--	--	52 µl
Calibrator	--	52 µl	--
Gently mix and Incubate at 37°C for 5 minutes			
Reagent 2	260 µl	260 µl	260 µl
Gently mix and Incubate at 37°C for 1 minutes, then measure the change of Optical Density per minute (ΔOD/min) over the next 2 minutes			

Calculation:

$$\text{Concentration} = \frac{\Delta Abs/\min_{Sample}}{\Delta Abs/\min_{Calibrator}} \times \text{Concentration of Calibrator}$$

Quality Control

All clinical laboratories should establish an internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the laboratory's QC program.

EXPECTED VALUES:

Adult: ≤15µmol/L
Elder population ≥ 60 years: 15 - 20µmol/L

Each laboratory should establish its own reference range. Amylase results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
This assay is linear up to 50µmol/L.
For samples with a higher concentration, dilute 1:1 with 0.9% NaCl (9g/l) and re-assay. Multiply result by 2.

Interfering substances: [5]
Results of study are as follows:

Bilirubin (mixed isomers): Less than 10% interference up to 600 µmol/l Bilirubin
Haemolysis: Less than 10% interference up to 500 mg/dl Haemoglobin
Lipemia: Less than 10% interference up to 500 mg/dl Lipemia

Sensitivity:
The Lower Detectable Level was estimated at 0.7µmol/L.

Precision:

Within Run N = 20	Mean (µmol/L)	*SD*	*% CV*	*Between Run N = 20*	Mean (µmol/L)	*SD*	*% CV*
Level 1	12.2	1.02	2.62	Level 1	12.9	0.96	2.68
Level 2	25.6	2.42	1.78	Level 2	26.4	3.84	1.92

Method Comparison:
Using 23 samples, a comparison, between this HCY test (y) and another commercially available test (x), gave the following results:

y = 0.97x – 3.67	r = 0.997	Sample range: 3 to 36µmol/L

BIBLIOGRAPHY:

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