HOMOCYSTEINE

Liquid Reagent

CE

In Vitro Diagnostic reagent for the quantitative determination of Homocysteine (HCY) in serum and plasma. Store at 2-8ºC .

REF: HCY-010B1 / HCY-010B2

Summary:

Homocysteine (Hyc) 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. 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 and osteoporosis.

Principle:

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+ (Δ A340 nm) is directly proportional to the concentration of homocysteine.

Serine + Homocysteine $\xrightarrow{CBS}$ L-cystathionine

L-cystathionine $\xrightarrow{CBL}$ Homocysteine+ Pyruvate + NH3

Pyruvate + NADH $\xrightarrow{LDH}$ Lactate + NAD+

Composition of the reagent(s):

R1 Enzymes: Tris Buffer, LDH - 35KU/L, L-Serine - 0.76 mmol/L, TCEP - 0.5 mmol/L, NADH - 0.47 mmol/L, PRESERVATIVES
R2 Enzymes: Tris Buffer, Cystathionine β-Synthase - 20KU/L, Cystathionine β-lyase - 10KU/L, L-Serinelyase - 20KU/L, PRESERVATIVES

Precautions:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.
Components Colour and Appearance:
Reagent 1: Clear, colourless liquid, Reagent 2: Clear, light yellow liquid.
Any significant changes could indicate that the assay might be compromised. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.
Safety precautions:
This product is not hazardous under EU specifications. Contains Sodium Azide. Material Safety Data Sheet is available upon request.
Handling precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.
- Carbamazepine, methotrexate, phenytoin, nitrous oxide, or 6-azauridine triacetate may affect the homocysteine concentration.

Instruments:

This assay is designed to run on clinical chemistry analysers. Refer to relevant user's manual or Laboratory internal practice for routine maintenance procedures. Instrument applications are available upon request.

Preparation:

- **R1 and R2** are ready to use.
Before use, mix reagent by gently inverting each bottle.
If stored and handled properly, unopened components are stable until the expiry date stated on the label.

Samples:

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.

Equipment:

- HCY Calibrator and Controls
- General Laboratory Equipment

Procedure:

1. Assay conditions:
Wavelength: . . . . . 340 nm
Cuvette: . . . . . 1 cm. light path
Temperature . . . . . .30°C or 37°C

2. Standard procedure:

	Blank	Calibrator	Sample
R1 (µL)	960	960	960
Sample (µL)	-	-	-
Calibrator (µL)	-	52	-

4. Gently mix and Incubate at 37°C for 5 minutes.
5. Add:

R2(µL)	260	260	260

6. Gently mix and Incubate at 37°C for 1 minute.
7. measure the change of Optical Density per minute (ΔOD/min) over the next 2 minutes.

Using recommended Calibrator, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Calculations:

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

Quality control:

All clinical laboratories should establish an Internal Quality Control program. Check 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.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At pre-established intervals following the Q.C. Laboratory recommandations.

Reference 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:
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:

	Intra-assay			Inter-assay		
N=20	Mean (µmol/L)	SD	%CV	Mean (µmol/L)	SD	%CV
level 1	12.1	1.02	2.62	12.9	0.96	2.68
level 2	25.6	2.42	1.78	2.4	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

References:

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2. Perry IJ, Refsum H, Morris RW, et al. Prospective Study of Serum Total Homocysteine Concentration and Risk of Stroke in Middle-aged British Men. The Lancet 1995;346:1395-1398
3. Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations:an expert opinion. Clin Chem 2004;50(1):3-32
4. Nehler MR, Taylor LM Jr, Porter JM. Homocysteinemia as a Risk Factor for Atherosclerosis: A Review. Cardiovascular Pathol 1997;6:1-9
5. Mudd SH, Levy HL, Skovby F. Disorders of Transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, et al., eds The Metabolic and Molecular Basis of Inherited Disease. New York: McGraw-Hill, 1995;1279-1327
6. Ueland PM, Refsum H, Stabler SP, et al. Total Homocysteine in Plasma or Serum: Methods and Clinical Applications. Clin Chem 1993;39:1764-1779

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