Homocysteine
Enzymatic Method

DIATEK

CLINICAL SIGNIFICANCE :

Homocysteine (Hcy) is a thiol-containing amino acid produced by the intracellular de methylation of methionine. Total homocysteine (tHcy) represents the sum of all forms of Hcy (including forms of oxidized, protein bound and free). Elevated level of homocysteine has emerged as an important risk factor in the assessment of cardiovascular disease. Elevated homocysteine levels are caused by four major factors, including genetic deficiencies in enzymes, involved in Hcy metabolisms such as cystathionine beta-synthase(CBS), methionine synthase (MS), and methylene-tetrahydrofolate reductase as well nutritional deficiency in B vitamins (B6, B12 and folate) and renal failure for effective amino acid clearance. Drug interactions such as nitric oxide, methotrexate and phenytoin that interfere with Hcy metabolisms. Elevated levels of tHcy are also linked with Alzheimer's disease and osteoporosis.

TEST PRINCIPLE :

The enzymatic test for the quantitative homocysteine determination is based on a series of enzymatic reactions causing a decrease in absorbance value due to NADH oxidation to NAD+. Hcy concentration in the sample is directly proportional to the quantity of NADH converted to NAD+. The enzymatic reactions are the following:

$$T(Hcy) \longrightarrow (Hcy)free$$

$$(Hcy)free + SAM \xrightarrow{\text{s-Methyltransferase}} Methionine + SAH$$

$$SAH + NADH \xrightarrow[\text{Glutamatedehydrogenase}]{\text{SAM Hydrolase + Adenosine deaminase}} Inosine + NAD+$$

Pack Size : 30 ml
R 1 (27.5ml) + R 2 (2.5 ml)

Active ingredient :

S-adenosylmethionine (SAM) 0.1 mM, NADH >0.2 mM TCEP >0.5 mM, 2-oxoglutarate 5.0 mM, Glutamate dehydrogenase 10 KU/L, SAH hydrolase 3.0 KU/L, Adenosine deaminase 5.0 KU/L, Hcy methyltransferase 5.0 KU/L, Tris Buffer

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

SAMPLES :

Fresh serum or heparinised plasma.
Collected blood specimens should be kept on ice and centrifuged within an hour. Hemolyzed or turbid specimens or severly lipemic specimens are not recommended for Hcy assay.

Stability: 4 weeks at 2-8 °C,

INTERFERENCES

An interference study was performed by testing a serum sample spiked with varied concentrations of endogenous substances. The following substances normally present in the serum produced less than 10% deviation when tested at the stated concentrations: 500µM NH_4Cl, 1 mM NaPi, 1 mM NaF, 2500 mg/dL Triglycerides, 20 mg/dL Bilirubin, 1200 mg/dL Hemoglobin, 0.5 mM* Glutathione,10 mM Ascorbic Acid, 1 mM L-Cysteine, 20 µM S- Adenosylmethionine (SAM), 100 µM** Adenosine, 100 µM** Cystathionine.

* Glutathione was originally tested at 1 mM level, the interference was +13.5%. When retested at 0.5 mM level, the interference was less than 10%.

** The concentrations tested are about 5-10 times higher than the normal range of serum levels.

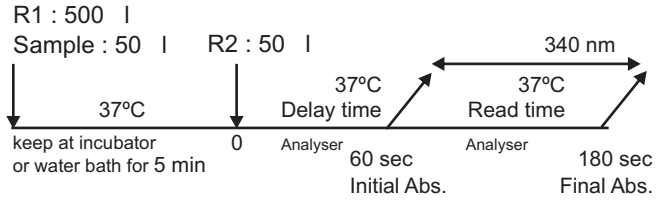
ASSAY CONDITIONS:

Wavelength : .....340 nm
Cuvette: .....1 cm light path
Constant temperature 37ºC
Reaction (Mode).....Fixed Time / Initial Rate / Two Point Kinetic
Number of Calibrator.....2
Calibrator Conc.....On the Label (Calibrator Vial)
Delay .....60 sec
Read time.....180 sec
Linearity.....50µmol/L
Unit.....µmol/L
Slope of reaction.....Decreasing

PROCEDURE :

1. Mix 50 µl sample / Calibrator with 500 µl R1 and incubate at 37ºC for 5 minutes at incubator or water bath.
2. Add 50 µl R2, mix and read initial absorbance after 60 sec. and measure final absorbance after 180 sec. against distilled water blank at 340 nm by an Analyser.
4. Calculate absorbance change per minute (△A/min).

Assay Procedure summary:



CALCULATIONS :

Concentration of Homocysteine (µmol/L) = $\frac{\textbf{A1 - A2 (Sample)}}{\textbf{A1 - A2 (Calibrator)}}$ X **Calibrator Conc.**

REFERENCE RANGE :

Adults : < 17 µmol/L
Age specific normal range may also be referred as per the studies published in Clin. Chem (1997) and Am. J. Hum.Genet.(1997)

Age		Hcy (µmol/L)
Newborns		3 - 9
Adolescents		5 - 11
Adults :	Male :	6 - 22
	Female:	3 - 18
Elderly (>60)		upto 25

The above reference range is guideline and all the laboratories must establish their own normal reference range. Final diagnosis should be made with correlation of clinical factors.

LINEARITY AND DETECTION LIMIT :

2.5 - 50 µmol/L.

The results of the performance characteristics depend on the analyzer used. If the results obtained were greater than linearity limit, dilute the sample 1 : 2 with NaCl 9 g/L and multiply the result by 2.

BIBLIOGRAPHY:

1. Eikelboom JW, et. al. Ann Intern Med 131:363-75, (1999)
2. Scott J, Weir D. Q J Med 89: 561– 3 (1996)
3. Nygard O, N Engl J Med. 337(4):230-6 (1997)
4. Seshadri S. et al. N. Engl. J. Med. 346:477-483 (2002)
5. McLean R. et al. N. Engl. J. Med. 350: 2042-2049 (2004)
6. Refsum H. Clinical Laboratory News May 2002, pp 2-14
7. Guttormsen AB et al. J Nutr. 124(10):1934-41 (1994)
8. Vilaseca et al. Clin. Chem. 43: 690-692 (1997)

DIATEK
(A unit of Sirus Biocare Pvt. Ltd.)
P-25, Kalindi Housing Scheme, Kalindi, Kolkata- 700089
Help line : 033 - 25223075