N BIO - HOMOCYSTEINE

(Enzyme cycling method)

KIT NAME	KIT SIZE	CAT. NO
N BIO - HCY	1 x 30 ml	DHCY01030M

GENUINE BIOSYSTEM

INTRODUCTION

Homocysteine is a sulphur-containing amino acid which is believed in causing arterial and venous atherothrombotic disease. It cannot be obtained from diet but only biosynthesized from methionine. In 1969 an American doctor indicated this relationship on patients with extremely elevated plasma Hcy concentration. A lot of studies also showed that it is associated with an increased risk of myocardial infarction and stroke because the elevated Hcy may reflects the increase in occurrence of blood clots.

METHOD PRINCIPLE

The Kit utilizes enzymatic and kinetic reactions to measure the amount of homocysteine (µmol/L) in human serum or plasma.

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

$$\text{L-cystathionine} \xrightarrow{\text{CBL}} \text{Homoctsteine} + \text{Pyruvate} + NH_3$$

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

Oxidized homocysteine is reduced to free homocysteine which reacts with serine by the catalysis of cystathionine β-synthase to produce L- cystathionine. L- cystathionine converts back to homocysteine in the presence of cystathionine β-lyase and pyruvate and ammonia are also formed in the same reaction. Pyruvate is then oxidazed to lactate catalyzed by lactate dehydrogenase to transfer hydrogen from $NADH^{+}$ to NAD. The process is quantified by measuring the absorabances at 340 nm in a kinetic fashion.

The rate of increase in absorbance at 340 nm is directly proportional to the amount of Hcy in the sample.

REAGENTS

Reagent Name	DHCY01030D
R1 HCY Reagent	1 x 22.5 ml
R2 HCY Reagent	1 x 7.5 ml
R3 HCY Calibrator	1 vial

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 14 days on board the analyser at 2-10°C. Protect from light and avoid contamination.

WORKING REAGENT PREPARATION AND STABILITY

Assay can be performed with use of separate R1-Hcy and R2-Hcy reagents or with use of working reagent. For working reagent preparation mix gently 3 parts of R1-Hcy with 1 part of R2-Hcy. Avoid foaming.

Stability of working reagent : 1 day at 2-8°C

CONCENTRATIONS IN THE TEST

Lactate dehydrogenase	>35 KU/L
Serine	0.76 mmol/L
NADH	0.47 mmol/L
Cystathionine β-synthase	>20% KU/L
Cystathionine β-lyase	>10 KU/L
EDTA	>0.1 g/L

SPECIMEN

Serum, it is recommended to perform test immediately after sample collection. If the test cannot be done immediately, specimens can be stored at 2~8°C for 48 hours.

PROCEDURE

These reagents may be used both for manual assay in several automatic analyzers. Applications for them are available on request.

Wavelength	340 nm
Temperature	37°C
Cuvette	1 cm

The determination can be also performed with use of separate R1-Hcy and R2-Hcy reagents.

Pipette into the cuvette:

Reagent	Calibrator(C)	Test (T)
R1 HCY Reagent	750 µl	750 µl
R2 HCY Reagent	250 µl	250 µl
R3 Calibrator	50 µl	-
Sample	-	50 µl

Mix well, after about 180 sec. (37°C) read the absorbance A1 of the test (T) and calibrator (C) against air or water. After exactly 120 secs. (for all temperature) read the absorbance A2 of the test (T) and calibrator (C). Calculate ΔA/min. (A1 - A2) for the test and calibrator.

CALCULATION

Hcy concentration = ΔA (T) / ΔA(C) x calibrator concentration

REFERENCE VALUES

4 to 15.4 µmol/L

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls

PERFORMANCE CHARACTERISTICS

- Linearity : upto 50 µmol/L (r ≥ 0.990)
- Precision : Within Run : Cv≤8%;
  Run-to-Run : Cv≤10%
- Reagent Blank Absorbance:
  at 340nm wavelength and 10 mm optical diameter, O.D. ≥1.0

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Bogdan N. Manolescu et al., Acta Biochimica Polonica 57(4):467-477 (2010)
2. Bruno Zappacostaetal., Clinical Biochemistry 39(1):62-66 (2006)
3. McCully K. S., Am J pathol. 56:111-128 (1969)

SYSTEM PARAMETERS

Method	Fixed time (2-point)
Wavelength	340 nm
Zero Setting	Distilled Water
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	----
Delay Time	180 secs
Read Time	120 secs
No. of Reading	3
Interval Time	----
Sample Volume	0.05 ml (50 ul)
Reagent Volume	1.0 ml (1000 ul)
Standard Concentration	Refer Calibrator vial
Units	µmol/L
Factor	----
Reaction Slope	Decreasing
Linearity	50 µmol/L

IVD CE

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