Homocysteine Test Kit

Enzymatic

Quantitative determination of Homocysteine in human Serum / Plasma / other body fluids. Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Pack Size
HCY 25	1 X 25 ml
HCY 30	1 X 30 ml
HCY 50	1 X 50 ml
HCY 100	1X100 ml
HCY 5000	1X5000 ml
HCY 10000	1X10000 ml

CLINICAL SIGNIFICANCE

Homocysteine (Hcy) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Total homocysteine (tHcy) represents the sum of all forms of Hcy including forms of oxidized, protein bound and free. Elevated level of tHcy has emerged as an important risk factor in the assessment of cardiovascular disease. Excess Hcy in the bloodstream may cause injuries 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 tHcy levels are caused by four major factors, including:

a) genetic deficiencies in enzymes involved in Hcy metabolisms such as cystathionine betasynthase (CBS), methionine synthase (MS), and methylenetetrahydrofolate reductase (MTH FR)

b) nutritional deficiency in B vitamins such as B6, B12 and folate;

c) renal failure for effective amino acid clearance, and

d) drug interactions such as nitric oxide, methotrexate and phenytoin that interfere with Hcy metabolisms.

METHODOLOGY & PRINCIPLE:

Method: Enzymatic.

In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, 5-adenosylmethionine (SAM) catalyzed by a Hcy S-methyltransferase to form methionine (the Hcy conversion product of Hcy) and S-adenosylhomocysteine (SAH, the co-substrate conversion product). SAH is assessed by coupled enzyme reactions including SAH hydrolase, adenosine (Ado) deaminase and glutamate dehydrogenase, wherein SAH is hydrolyzed into adenosine (Ado) and Hcy by SAH hydrolase. The formed Hcy that is originated from the co-substrate SAM is cycled into the Hcy conversion reaction by Hcy Smethyltransferase. This forms a co-substrate conversion product based enzyme cycling reaction system with significant amplification of detection signals. The formed Ado is immediately hydrolyzed into inosine and ammonia which reacts with glutamate dehydrogenase with concomitant conversions of NADH to NAD+. The concentration of Hcy in the sample is indirectly proportional to the amount of NADH converted to NAD+.

REAGENT PREPARATION

R1, and R2 are ready-to-use liquid stable reagents. Calibrators and controls are ready-to-use stable liquids.

REAGENT STORAGE AND STABILITY

Unopened, avoid light preservation in 2 ~ 8 ℃, valid for 12 months; Opened, avoid light preservation in 2 ~ 8 ℃, valid for 1 month. Reagent is not allowed frozen.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

It is best to fresh serum or heparin anticoagulant blood plasma, once take, blood immediately centrifugal separation of plasma.

Sample stability: 2-8℃ preservation stability in 2 weeks.

ASSAY PROCEDURE

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, Calibrator and samples to room temperature 18 - 28ºC, prior to analysis.

AUTOMATED PARAMETERS	
Temperature	37°C
Cuvette light path	1.0cm
Primary Wavelength	340 nm
Secondary Wavelength	405 nm
Direction	Decreasing
Sample Vol	13 µL
Reagent Vol	305 µL
Delay Time	90 seconds
Read Time	180 seconds
Linearity	0～50µmol/L

MULTI POINT CALIBRATION

Prepare the following HCY calibrator dilutions in NaCl 9 g/dL, Multiply the concentration of the HCY calibrator by the corresponding factor stated in the table below to obtain the HCY concentration of each dilution.

Calibrator Dilution	1	2	3	4	5
Calibrator HCY (µl)	-	25	50	75	100
NaCl 9 g/dL (µl)	100	75	50	25	-
Factor	0	0.25	0.50	0.75	1.0

MANUAL ASSAY PROCEDURE

Addition Sequenc	Calibr (C)	Test (T)
Reagent 1	240 µL	240 µL
Calibrator	13 µL	-
Sample	-	13 µL
Mix well and incubate for 5 minutes at 37º C		
Reagent 2	65 µL	65 µL

Mix well, and read the absorbance after 90 sec A1 and after 180 sec minutes A2 of the sample/calibrator addition.

CALCULATION

Results are calculated, usually automatically by the instrument, as follows:

Sample Concentration = Sample ΔAbs/min ×Calibrator Concentration

Calibrator ΔAbs/min

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
HCY Level 2	12.83	0.15	1.18%
HCY Level 3	25.60	0.28	1.09%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
HCY Level 2	12.52	0.07	0.52%
HCY Level 3	25.64	0.20	0.79%

LINEARITY

The maximum linearity is 50µmol/L. If testing results is upper limit, dilute with 0.9% sodium chloride solution before test, results multiplied by the dilution ratio.

Limit of detection: 0 µmol/L

METHOD COMPARISON

A comparison of Homocysteine with a commercially available assay (x) using 20 samples gave following results: $R^2 = 0.9900$

REFERENCE VALUES

In plasma, Hcy age-related in the normal reference range,
adult ≤15µmol/L,
Above 60 years 15~20µmol/L,
Above 100 years
25~27µmol/L.
The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

LIMITATION OF THE PROCEDURE

HCY testing is just one of the standard that clinicist diagnose the patient. Clinical physicians should according to patients' bodies, history and other diagnostic program, to get comprehensive judgment.

INTERFERENCE

- Hemoglobin: No interference found upto 0.5 mmol/l.
- Bilirubin: No interference found upto 1200 mg/dl
- Ascorbic Acid: No interference found upto 1 mmol/l.
- These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used.

BIBLIOGRAPHY

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GLOSSARY OF SYMBOL

	Consult Instruction for Use
	Catalog Number
	Store between
	Manufacturer
	Keep away from sunlight



India office
BIOBOON BIOTECH INDIA LLP
207. Regent Square
2nd floor, Above D'mart shopping mall
Adajan
Surat-395009
Email : info@bioboonbiotech.com

USA Address:-
BIOBOON BIOTECH LLC
1526 West 3rd Street,
Plainfield, NJ, 07063
USA
Email : mahipal@bioboonbiotech.com