Homocysteine Assay Kit (HCY)

Method: Enzymatic

Cat. No.	Size	Instrument
GB180Z2	R1:2×20ml R2:1×13ml	or Hitachi 7150 Shimadzu CL7200/8000
GS181Z2	R1:2×20ml R2:1×13ml	or Olympus AU640/400/600

INTENDED USE

Enzymatic homocysteine assay is intended for the *in vitro* quantitative determination of total L-homocysteine in serum or plasma.

The assay can assist in diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria. The assay is not intended for correlating B_{12} or folate with homocysteine levels.

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[1,2,3]. Excess Hcy in the blood stream 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 beta-synthase(CBS), methionine synthase (MS), and methylenetetrahydrofolate reductase (MTHFR); **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.

Elevated levels of tHcy are also linked with Alzheimer's disease[4] and Osteoporosis[5]. Guidelines for tHcy determination in clinical laboratories have recently been established[6].

PRINCIPLE

Enzymatic tHcy assay is based on a novel assay principle that assesses the co-substrate conversion product (a molecule that is not a substrate of the Hcy conversion enzyme, and does not contain any element from sample Hcy) instead of assessing co-substrate or Hcy conversion products of Hcy as described in the literature. In this assay, oxidized Hcy is reduced to free Hcy which then reacts with a co-substrate, S-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 S-methyltransferase. 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^+ (A340nm).

SPECIMEN COLLECTION

Fresh serum or heparin plasma are the recommended samples for the Hcy assay. EDTA plasma samples can also be used. It is important to centrifuge blood samples immediately after collection to separate the plasma from the blood cells. If immediate centrifugation is not possible, collected blood specimens should be kept on ice and centrifuged within an hour. Hemolysed or turbid specimens or severely lipemic specimens are not recommended for Hcy assay. After separation of plasma from cells, Hcy is stable for at least 4 days at room temperature and stable for several weeks at 0-8°C, and stable for several months or years at -20℃[7].

REAGENT COMPOSITION

Contents	Concentration
S-adenosylmethionine (SAM)	0.1 mmol/L
NADH	> 0.2 mmol/L
TCEP	> 0.5 mmol/L
2-oxoglutarate	5.0 mmol/L
Glutamate dehydrogenase	1 0 KU/L
SAH hydrolase	3.0 KU/L
Adenosine deaminase	5.0 KU/L
Hcy methyltransferase	5.0 KU/L

STABILITY AND PREPARATION OF REAGENTS

BSBE Hcy assay reagents, calibrators, and controls should be stored at 2-8℃. DO NOT FREEZE. The reagents, calibrators, and controls are stable when stored as instructed

Beijing Strong Biotechnologies, Inc.
Add: 5/F Kuang Yi Building, No. 15 Hua Yuan Dong Lu, Haidian District, Beijing 100191 P. R. China
Tel: +86 10 8201 2486 Fax: +86 10 8201 2812
Web: www.bsbe.com.cn Email: jg.tech@bsbe.com.cn

CE Revised 01FEB11 version 1102

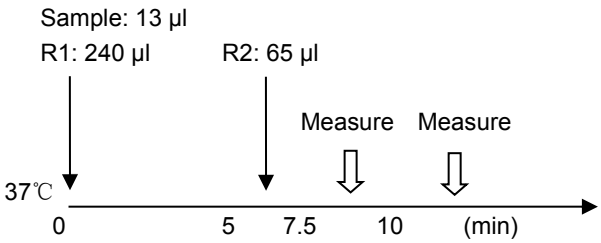
until the expiration date on the label. Do not mix reagents of different lots.

STABILITY AND PREPARATION OF REAGENTS

R1 and R2 Contents supplied ready for use. Stable up to expiry date when stored at +2 to +8℃. (DO not freeze.)

ASSAY PROCEDURE

Main wavelength: 340 nm
Second wavelength: 700 nm



CALIBRATION

Use the provided calibrators for calibration.
The calibration curve is stable for at least five days.

QUALITY CONTROL

Quality control materials are intended for use only to monitor accuracy and precision. The values for these controls should fall within specified limits. If the control values fall outside these ranges and repetition precedes technical error the following steps should be taken:

1. Check wavelength setting and light source
2. Ensure that cuvettes and glassware in use have been thoroughly cleaned
3. Check water, contaminants eg bacterial growth may contribute to inaccurate results
4. Check that assay temperature is accurate
5. Ensure that the reagent pack contents are still within expiry date
6. Contact Beijing Strong Biotechnologies Technical Support, Tel (+86 10) 61667168

NORMAL RANGES

In most of the clinical laboratories, 15 µmol/l is used as the cut-off value for normal level of Hcy for adults.[8,9] However, each laboratory is recommended to establish a range of normal values for the population in their region.

MAIN PERFORMANCE CHARACTERISTICS

LIMITATIONS:

1. The measuring range of the assay is from 1.5 to 50 µmol/L.
2. The reagent should be clear. It should be discarded if it becomes turbid or the initial absorbance is less than 0.5 at 340 nm (light path 0.6 cm).
3. S-adenosylhomocysteine (SAH) will cause a significant positive interference. However, SAH
4. is either not detectable or at sub-nmole/l concentrations in normal plasma, and should not cause concern.
5. Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azuridine triacetate may have higher levels of Hcy due to metabolic interference with Hcy metabolism.

PRECISION

Inter Precision	Level 1 (7 µmol/L)	Level 2 (12 µmol/L)	Level 3 (29.5 µmol/L)
number	20	20	20
CV	4.5%	1.87%	2.4%

Intra Precision	Level 1 (7 µmol/L)	Level 2 (12 µmol/L)	Level 3 (29.5 µmol/L)
number	20	20	20
CV	5.87%	4.88%	2.57%

SPECIFICITYN/ITERFERENCE[5]

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 with the following stated concentrations:

Hemoglobin 500 mg/dl
Bilirubin 40 mg/dl
ascorbic acid 10mM
Triglyceride 2500 mg/dl

Performance Characteristics assay range

Results are printed out in µmol/L. Note: Samples with values greater than 50 µmol/L should be diluted 1:2 with water and rerun. Multiply results by 3.

SENSITIVITY

The minimum level of Total homocysteine detectable with an acceptable level of precision has been determined as 1.5 µmol/L.

METHOD COMPARISON

The BSBE method (X) was compared to another commercially available method (Y). 40 patients were tested. Linear regression analysis of the data resulted in the following equation:

Y = 0.94X+1.05
R^2= 0.99

REFERENCES

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)
9. Faure-Delanef et al. Am. J. Hum. Genet. 60: 999-1001(1997)

INDEX OF SYMBOLS

	Manufacture
	Catalogue Number
	Lot number
	Date of manufacture
	Use by(Expiration date)
	For In-Vitro Diagnostic use only
	Stored at 2-8℃
	Attention:See instruction for use
	Authorized Representative in the European Company

Beijing Strong Biotechnologies, Inc.
Add: 5/F Kuang Yi Building, No. 15 Hua Yuan Dong Lu, Haidian District, Beijing 100191 P. R. China
Tel: +86 10 8201 2486 Fax: +86 10 8201 2812
Web: www.bsbe.com.cn Email: jg.tech@bsbe.com.cn