FORMOSA BIOMEDICAL TECHNOLOGY CORP.

IVD For *In Vitro* Diagnostic

Cat No：BC-0003B

TOTAL HOMOCYSTEINE BIOCHEMICAL ASSAY KIT

INTENTED USE

The Formosa Biomedical Technology Total Homocysteine Biochemical Assay Kit is an *in vitro* assay for the quantitative determination of total homocysteine in plasma.

INTRODUCTION

Over 1 million of Americans died of cardiovascular disease (CVD) such as heart attack or stoke. It was long thought that CVD is related to the increased level of cholesterol in the blood , but yet 25% patients with heart attack have no obviously elevated blood cholesterol level or other risk factors been observed. However, the metabolism of homocysteine (HCY) is found to be related to the CVD or stoke in recent study[1-3].

Homocysteine is a thiol-containing amino acid produced by the intracellular demethylation of methionine. HCY is exported into plasma where it circulates mostly in its oxidized forms bound to plasma proteins. Smaller amounts of reduced homocysteine and disulfide homocystin (HCY-SS-HCY) are present. Total homocysteine (tHCY) represents the sum of all HCY species found in plasma and serum. HCY is either metabolised to cysteine or to methionine. In the vitamin B6 dependent transsulphuration pathway HCY is irreversibly catabolized to cysteine. A major part of HCY is remethylated to methionine, mainly by the folate and cobalamin-dependent enzyme methionine synthase. HCY accumulates and is excreted into the blood when these reactions are impaired[4-5]. The elevated concentration of homocysteine in the blood is considered an independent risk factor of CVD.

Epidemiological studies have investigated the relationship between HCY levels in blood and CVD. A meta analysis of 27 epidemiological studies, including more than 4000 patients, estimated that a 5 μM increase in HCY was associated with an odds ratio for coronary artery disease (CAD) of 1.6 for men and 1.8 for women. Peripheral arterial disease also showed a strong association[6].

Certain patient groups with anemia and/or asthenia also demonstrate increased levels of plasma or serum HCY. Patients with chronic renal disease experience an excess morbidity and mortality due to arteriosclerotic CVD. Elevated concentration of HCY is a frequently observed finding in the blood of these patients. Although such patients may lack some of the vitamins involved in the metabolism of HCY, the increased levels of HCY are mainly due to impaired removal of HCY from the blood by the kidney[7].

Severely elevated concentrations of HCY are found in subjects with “homocystinuria”, a rare genetic disorder of the enzymes involved in the metabolism of HCY. Patients with homocystinuria exhibit mental retardation, early arteriosclerosis and arterial and venous thromboembolism[8]. Drugs such as methotrexate, carbamazepine, phenytoin, nitrous oxide and penicillamine interfere with the HCY metabolism and may give elevated levels of HCY[9].

PRINCIPLE OF THE METHOD

The Formosa Biomedical Technology Total Homocysteine Biochemical Assay is an enzymatic method which measures total homocysteine in plasma. Bound HCY is reduced to free HCY which then catalyzed by recombinant methionine α,γ-lyase (HCY enzyme) to produce H_2S. The H_2S subsequently reacts with a chromophore, and the product is measured optically at 660nm. The optical density is proportional to the tHCY level.

KIT COMPOSITION

1. Reagent 1 Buffer:	Tris buffer	9mL×10
2. Reagent 2:	Oxidant	18mL×1
3. HCY Enzyme:	Methionine α,γ-lyase	10vials
4. Reducing reagent:	DTT	10vials

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Not to be used internally in humans and animals.
- The assay should be performed by the doctor or the trained medical technician.
- Normal precautions for handling laboratory reagents should be followed.
- Avoid contact with skin and eyes. If the reagents come in contact with skin or eyes, rinse immediately with water. Consult a physician if necessary.
- Do not use reagents past the expiration date stated on each reagent container label.
- Do not mix or use reagents from one test kit with those from different lot number.
- Reagents contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. Flush drains with large amount of water when discarding the reagents.
- Additional safety information concerning storage and handling of this kit is provided within the Material Safety Data Sheet for this kit.

STABILITY AND STORAGE

All reagents should be stored at 2-8℃. Unopened reagents are stable until the expiration date on the label. Once opened, store tightly capped at 2-8℃ and use within 30 days. **Reconstituted reagent 1 should be used within 48 hours.**

SPECIMEN COLLECTION AND PREPARATION

- Fresh EDTA-plasma free from homolysis or turbidity is recommended for homocysteine determination.
- Overnight fasting is recommended before blood is drawn.
- Place all specimens on ice after collection and prior to processing, and separate plasma from the blood cells by centrifugation for up to 6 hours.
- The plasma samples may be stored at 2-8℃ for two weeks.
- The plasma samples should be stored at -20℃ for long term storage.

PROCEDURE

Materials supplied

Refer to the section entitled “KIT COMPOSITION”.

Materials required but not supplied

Hitachi 917 Automatic Analyzer

Pipets

Reagent preparation

Reagent 1 should be prepared freshly before use.

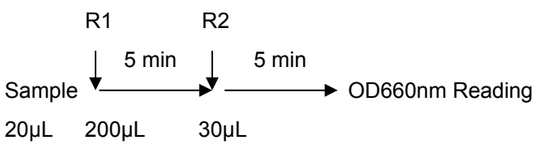
- Open a foil bag which contains one vial of HCY Enzyme and one vial of Reducing reagent. Draw out the vial of Reducing reagent and add about 2mL Reagent 1 Buffer into it. Replace the stopper immediately and gently mix by inversion several times. Let it stand for 1 minute. Transfer the reconstituted solution back to the original Reagent 1 buffer bottle. Recap and gently mix the reconstituted

solution by inversion several times. Take 2mL reconstituted solution back to the vial of Reducing reagent. Replace the stopper immediately and rinse the vial by inversion several times. Transfer the rinsed solution back to the reconstituted solution. Draw out the vial of HCY Enzyme and add about 2mL reconstituted solution into it. Replace the stopper immediately and gently mix by inversion several times. Let it stand for 1 minute. Transfer the reconstituted solution back to the original Reagent 1 buffer bottle. Recap and gently mix the reconstituted solution by inversion several times. Take 2mL prepared R1 back to the vial of HCY Enzyme. Replace the stopper immediately and rinse the vial by inversion several times. Transfer the rinsed solution back to the prepared R1. Let it stand for 10 minutes. Mix the prepared R1 well prior to use. Avoid foaming during the preparation. The fresh prepared R1 is available for 30 tests.

- **The prepared R1 is stable within 48 hours. Once past 48 hours, discard the remaining R1.**

Assay procedure

An example of standard protocol automated application:



Parameter for Hitachi 917 is available on request.

RESULTS

The tHCY levels are calculated and printed by the automatic analyzer in μM.

QUALITY CONTROL AND REFERENCE RANGE

A quality control program is recommended for all clinical laboratories. The analysis of control material in both normal and abnormal ranges with each assay is recommended for monitoring the kit performance. The range of acceptable control limits should be established by individual laboratories. The currently acceptable normal reference range is 5-15μM.

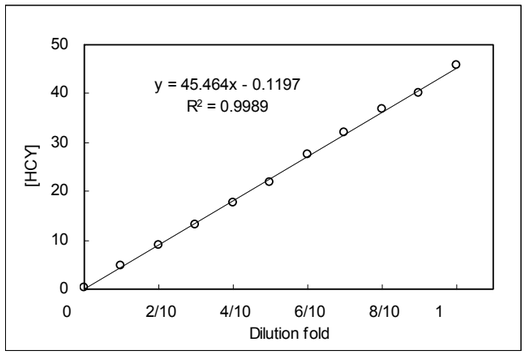
LIMITATIONS OF THE PROCEDURES

- Follow the suggested procedure will result in the confident determination, especially the plasma sample preparation and the Reagent 1 preparation.
- Hemolytic, icteric or lipemic samples may interfere the assay.

PERFORMANCE CHARACTERISTICS

The following performance data was obtained using a Hitachi 917 analyzer

DILUTION LINEARITY



PRECISION

Within-run precision

	Sample 1	Sample 2
Mean	10.4	20.8
S.D.	0.2	0.4
C.V.	1.9%	2.0%
Min.	10.2	19.8
Max.	10.7	21.2
Range	0.5	1.4
N	10	10

Between-run precision

	Sample 1	Sample 2
Mean	10.8	22.7
S.D.	0.3	0.6
C.V.	2.6%	2.6%
Min.	10.4	21.5
Max.	11.4	24.0
Range	1.0	2.5
N	22	22

LOWER LIMIT of DETECTION

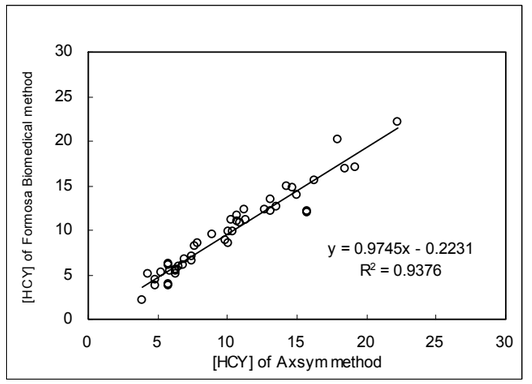
The lower limit of detection of this methods is estimated to be ＜0.5μM.

FUNCTIONAL SENSITIVITY

The functional sensitivity of this methods is estimated to be ＜0.5μM.

CORRELATION of METHODS

N=45



REFERENCE

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9. 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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