# LiquiMAX HOMOCYSTEINE

**Enzymatic Method**

**ORDERING INFORMATION:**

| Ref. No. | Pack Size | Presentation |
|---|---|---|
| AVHCY - 22 | 22 ml | Two Liquid Reagents with Two Level Calibrators |
| AVHCY - 44 | 44 ml | |

**INTENDED USE:**

LiquiMAX Homocysteine is an in-vitro diagnostic kit is use for the quantitative determination of Homocysteine in human serum or plasma on photometric systems.

**PRODUCT FEATURES:**

1. Results correlate with Immuno Assays
2. Liquid Stable, Ready to use Two Reagents
3. 2 Calibrators Provided
4. 4 Minutes Non Linear Fixed Time procedure (60 Sec Delay+180 Sec Measuring)
5. Linearity : 50 µmol/L
6. Measuring Wavelength 340 nms
7. Calibrators are standardized to NIST SRM 1955 (Homocysteine Standard Reference Material)
8. Available as multipurpose reagents and dedicated system packs

**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. 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 homocysteine levels are caused by four major factors, including:

1. genetic deficiencies in enzymes involved in Hcy metabolisms such as cystathionine beta-synthase(CBS), methionine synthase (MS), and methylenetetrahydrofolate reductase(MTHFR);
2. nutritional deficiency in B vitamins (B6, B12 and folate);
3. Renal failure for effective amino acid clearance;
4. 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. Guidelines for tHcy determination in clinical laboratories have recently been established.

**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 arethe following:

$$T(HCY) \xrightarrow{\text{s-Methyltransferase}} (HCY)free$$

$$(HCY)free + SAM \xrightarrow{\text{SAM Hydrolase + Adenosine deaminase}} Methionine + SAH$$

$$SAH + NADH \xrightarrow[\text{Glutamatedehydrogenase}]{} Inosine + NAD^{+}$$

**STORAGE AND STABILITY**

Unopened kit components: Up to the expiration date at 2-8°C.
If the reagent is opened and stored in automatic chemistry analyzer, the reagent can be stored for 2 week.

**KIT COMPONENTS**

1. Reagent R1
2. Reagent R2
2. HCY Calibrators (2) : Concentration as stated on the label

**COMPOSITION**

| Active Ingredients | Concentration |
|---|---|
| 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 | 5.0KU/L |
| Hcy methyltransferase | 5.0KU/L |

**REAGENT RECONSTITUTION & STABILITY**

Reagent are liquid stable no need for reconstitution.
When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

**MATERIAL REQUIRED BUT NOT PROVIDED**

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

**REAGENT DETERIORATION**

Discard any turbid reagent if blank reagent absorbance less than 0.8 at 340 nm against distilled water.

**WARNING & PRECAUTIONS**

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

**SPECIMEN COLLECTION & STORAGE**

A minimum of 8 hours fasting is required for Specimen Collection as the Amino Acid Methionine rich food intake grossly elevate the real homocysteine levels.
1) EDTA OR Heparin Plasma. 2) Preferably Serum collected in Serum Separator Tubes (SST) Or Serum collected in simple tubes.
It is important to centrifuge blood samples immediately after collection to separate the serum from the clotted blood.If immediate centrifugation is not possible, 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: 7 days at 2-8 °C,
4 weeks at - 20 °C.

**SYSTEM PARAMETERS :**

| | | |
|---|---|---|
| Calibrator Type | : | Multi Point Calibration |
| Reaction Type | : | Fixed Time / Two Point / Multi Standard/ Non Linear |
| Reaction Direction | : | Decreasing |
| Sample Volume | : | 50 µl |
| Working Reagent Volume | : | R1 500 µl + R2 50 µl |
| Wave Length | : | 340nm |
| Calibrators Conc. | : | On the label (Lot Specific) |
| Flow Cell Temp. | : | 37°C |
| Linearity | : | 50 µmol/L |
| Zero setting with | : | Distilled Water |
| Delay | : | 60 Sec (A1) |
| Measuring | : | 180 Sec (A2) |

## TEST PROCEDURE

| Reagent | Calibrator | Sample |
|---|---|---|
| Reagent 1 | 500 µl | 500 µl |
| Calibrators (1, 2) | 50 µl | ---- |
| Sample | ----- | 50µl |
| Mix. Incubate for 5 minutes at 37°C. | | |
| Reagent 2 | 50µl | 50µl |

Mix. Incubate for 60 Seconds (Delay) at 37°C and read the absorbance (A1) 180 Seconds later read the absorbance (A2).

## CALCULATIONS :

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

## EXPECTED VALUES:

We have tested HCY activity in 350 indian human samples ( Sera ) of different age groups and the following reference ranges were drawn out of HCY assay. In most of the clinical laboratories, 22 µmol/L is used as the cut off value for normal level of HCY for adults.
According to the studies published in Clin.Chem. (1997) and Am. J. Hum. Genet. (1997) and the studies there of.

| Age | | HCY (µmol/l) |
|---|---|---|
| Newborns | | 3 – 9 |
| Adolescents | | 5 – 11 |
| Adults: | Male | 6 – 22 |
| | Female | 3 – 18 |
| Elderly (> 60) | | Up to 25 |
| Centenarians | | Up to 29 |

Marginal elevation of HCY values in adults may be attributed to B- Complex Deficiency where the clinician should clinically evaluate looking at the other qualifying reasons like disease history while arriving at the correct diagnosis

However, each laboratory is recommended to establishes a range of normal values for the population in their region.

## QUALITY CONTROL & CALIBRATION

HCY Controls are recommended for daily quality control. The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

## PERFORMANCE CHARACTERISTICS

### 1. Linearity

Linearity : 50 µmol/L

### 2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 2.5 µmol/L

### 3. 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.

### 4. Precision:

**Intra-Assay**

| Control | Mean (µmol/L) | SD (µmol/L) | CV% |
|---|---|---|---|
| Level 1 | 21.87 | 0.43 | 1.97 |
| Level 2 | 30.71 | 0.39 | 1.27 |
| Level 3 | 40.12 | 0.44 | 1.10 |

**Inter-Assay**

| Control | Mean (µmol/L) | SD (µmol/L) | CV% |
|---|---|---|---|
| Level 1 | 22.04 | 0.76 | 3.47 |
| Level 2 | 31.20 | 0.65 | 2.07 |
| Level 3 | 41.50 | 0.98 | 2.37 |

### 5. Method Comparison:

A comparison of Homocysteine determination using the LiquiMAX HCY (y) versus with another commercially available method (x) gave the following correlation (µmol/L): $y = 0.94x + 1.05$, $r = 0.9$,
Number of samples measured: 40

## LIMITATIONS

**2.5 - 50 µmol/L.**
Samples above this concentration should be diluted 1+1 with 0.9% NaCl solution and the result multiplied by 2.

## WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

## NOTES

**General precautions:**

For in vitro diagnostic use only.
Diagnosis should only be made after taking clinical symptoms and the results of other tests into consideration.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.

## REFERENCES:


1. McCully KS. Vascular Pathology of Homocysteinemia:Implications for the Pathogenesis of Arteriosclerosis. Am J Pathol 1969;56:111-122.
2. Malinow MR. Plasma Homocyst(e)ine and Arterial Occlusive Diseases:A Mini-Review. Clin Chem 1995;41:173-176


## Symbols Used on Pack

| | | | |
|---|---|---|---|
| REF | Catalogue Number | | Warning/Caution |
| LOT | Batch No. | IVD | In vitro diagnostic device |
| | Manufacturing Date | | Storage Limit |
| | Expiry Date | | Consult instruction for use |
| | Manufacturer | | Keep away from sunlight |
| | Keep Dry | | Do not use if package is damaged |
| | Contains sufficient no. of test | | |

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