Pariksha Biotech
A game changer in IVD

Homocysteine Estimation Kit

High-Q Homocysteine

Enzymatic Recycling Method

HIGH®
Command on Quality

Intended Use:
Kit for the quantitative determination of Homocysteine in human serum or plasma on photometric systems.

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 methylene tetra hydrofolate 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) ——————————> (Hcy) free
s-Methyltransferase

(Hcy) free + SAM ——————————> Methionine + SAH
SAM Hydrolase + Adenosine deaminase

SAH+NADH ——————————Inosine + NAD+
Glutamate dehydrogenase

Reagent Composition:

Active Ingredients	Concentration
S-Adenosylmethionine (SAM)	0.2 mM
NADH	>0.2 mM
TCEP	>0.6 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

Storage and stability
Reagents are stable till the expiry date mentioned on the labels when properly stored at 2-8°C.

Linearity:
The assay is linear up to 100 µmol/L HCY.

Specimen Preparation:
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 samples must not be used for Homocysteine estimation as they give grossly elevated results

Turbid specimens or severly lipemic specimens are not recommended for Hcy assay.

Stability of the specimen — 7 days at 2-8 °C, 4 weeks at - 20 °C.

Procedure Parameters: Two Level Calibration:

Reagent	Calibrator	Sample
Reagent 1	500 µl	500 µl
Calibrators (1,2)	30 µl	----
Sample	-----	30 µl

Mix. Incubate for 5 minutes at 37°C. Then add:

Reagent 2	50 µl	50 µl

Mix. Incubate for 30 Seconds (Delay) at 37°C and read the absorbance (A1) 180 Seconds later read the absorbance (A2).

Calculations:

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

System Parameters:

Calibration Method	:	Multi Standard/ Linear
Reaction Type	:	Fixed Time / Two Point
Reaction Direction	:	Decreasing
Sample Volume	:	30 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	:	100 µmol/L
Zero setting with	:	Distilled Water
Delay	**:**	**30 Sec**
Measuring	**:**	**180 Sec**

Quality control:
High-Q 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.

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Performance characteristics:

Measuring range:
2.5 - 100 µmol/L.
Samples above this concentration should be diluted 1+1 with 0.9% NaCl solution and the result multiplied by 2.

Reference 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.

Method comparison:
A comparison of the homocysteine determination using the High-Q 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

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.

Precautions for measurement:
Specimens should be treated as potentially infectious (HIV, Hepatitis B virus, Hepatitis C virus, etc.) and handled with appropriate caution.
Reagents with different lot numbers should not be interchanged or mixed.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Material safety data sheet available for professional user on request.

Interference:
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.

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 Homocysteine and Arterial Occlusive Diseases: A Mini-Review. Clin Chem

Ordering information:

Cat No:	Pack Size	Presentation
P-HCY - 20	20 ml	Two Liquid Reagents
P-HCY - 40	40 ml	with 2 Level Calibrators

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Product Features

- Liquid Stable, Ready to use Two Reagents
- 2 Calibrators Provided
- 3 Level Controls Provided (Optional)
- 3.5 Minutes Fixed Time, Multi Standard Linear procedure (30 Sec Delay+180 Sec Measuring)
- Linearity 100 µmol/L
- Measuring Wavelength 340 nms
- Standardized to NIST SRM 1955 (Homocysteine Standard Reference Material)
- Available as multipurpose reagents and dedicated system packs
- Results correlate with Immuno Assays

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

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Manufactured in India by :
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