Homocysteine

Homocysteine Reagent Test Kit

Intended Use

Diagnostic reagent for in vitro quantitative determination of Homocysteine in human serum or plasma on photometric analyzers.

Reagent Kits

Item Code	Pack size	
136619934841	R1 : 2 x 18.5 mL;	R2 : 2 x 5 mL

Clinical Significance

Homocysteine (Hcy) is a thiolcontaining 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 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 and osteoporosis. Guidelines for tHcy determination in clinical laboratories have recently been established.

Assay Principle

The DiaSys Homocysteine 2 Reagent Enzymatic 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 first reduced to free Hcy which then reacts with a co-substrate, Sadenosylmethionine (SAM) catalyzed by a Hcy Smethyltransferase to form methionine (the Hcy conversion product of Hcy) and S-adenosylhomocysteine (SAH, the co-substrate conversion product). SAH is assessed by oupled enzyme reactions including SAH hydrolase, adenosine (Ado) deaminase and glutamate dehrogenase, where in 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 Sethyltransferase. 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. In the last step, the enzyme glutamate dehydrogenase (GLDH) catalyzes the reaction of ammonia with 2-oxoglutarate and NADH to form NAD+. The concentration of Hcy in the sample is directly proportional to the amount of NADH converted to NAD+ (340nm).

Materials Required but not Provided

An analyzer capable of dispensing 2 reagents and measuring absorbance at 340 nm with temperature control (37° C).

Calibrators are sold separately

Controls are sold separately.

Reagent 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	5.0 KU/L
Hcy methyltransferase	5.0 KU/L

Reagent Preparation

The DiaSys Homocysteine 2 Reagent Enzymatic assay reagents are ready-to-use liquid stable reagents. Calibrators and controls are ready-to- use stable liquids.

Reagent Stability and Storage

The DiaSys Homocysteine2 Reagent Enzymatic assay reagents, calibrators, and controls should be stored at 2-8° C. **DO NOT FREEZE**. The reagents, calibrators, and controls are stable when stored as instructed until the expiration date on the label. Do not mix reagents of different lots.

Specimen Collection and Handling

Fresh serum, heparin plasma, or EDTA plasma can be used in the Hcy assay. 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 iceb and centrifuged within an hour. Hemolysed or turbid specimens or severely lipemic specimens are not recommended for DiaSys Hcy assay. After separation of plasma from cells, Hcy is stable for at least 4 days at room temperature, stable for several weeks at 0-8° C, and stable for sever- al months or years at –20° C.

Precautions

The reagents are for *in vitro* diagnostic use only. **DO NOT INGEST**. Avoid contact with skin and eyes. Contains sodium azide, which may react with lead or copper plumbing to form explosive compounds. Flush drains with copious amounts of water when disposing of this reagent.

Calibrators and controls are human serum based. Reagents contain glycerol as a stabilizer. Automated chemistry analyzers use on-board routine wash steps to prevent reagent carry-over by reagent probes. However, the efficiency of the routine reagent probe wash varies and additional wash steps may be needed. Please refer to the instrument operator manual. Specimens containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength 340 nm, Hg 334 nm

Optical path 1 cm

Temperature 37°C

Measurement Against reagent blank

	Blank	Sample or Calibrator
Sample or Calibrator	--	13µl
Dist.Water	13µl	--
Reagent 1	240 µl	240 µl
Mix,incubate for approx.. 5 mins at 37 °C and read absorbance (A1) then add:		
Reagent 2	65 µl	65 µl
Mix,incubate for 5 min. at 37 °C and read absorbance (A2)		

Δ A = (A2-A1) sample or calibrator.

Calibration

For analyzers, use Calibrators 1-5 for calibration.
The calibration curve is stable for at least five days.

Quality Control

We recommend that each laboratory use Hcy controls to validate the performanceof Hcy reagents. A set of normal and abnormal ranges of Hcy controls is available from DiaSys Laboratories. The range of acceptable control limits should be established by individual laboratories.

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

Reference Range
In most of the U.S. clinical laboratories, 15 mol/L is used as the cut-off value for normal level of Hcy for adults.8-9 In Europe, 12 mol/L is used as the cut-off value. However, each laboratory is recommended to establish a range of normal values for the population in their region.

Limitations
- The measuring range of the assay is from 3 to 50 mol/L. Samples with Hcy values higher than 50 mol/L should be diluted 1:1 with water.
- 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).
- Sadenosylhomocysteine (SAH) will cause a significant positive interference. However, SAH is either not detectable or at subnmole/L concentrations in normal plasma, and should not cause concern.
- 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.
- Addition of 3-deazaadenosine to inhibit Hcy production in red cells has been suggested. However, the DiaSys Hcy assay can not use samples containing 3-deazaadenosine since it inhibits one of the key enzymes used in the assay.

Performance Characteristics

Limit of Detection
To demonstrate the limit of detection (LOD) of the DiaSys Homocysteine 2 Reagent Enzymatic Assay, Homocysteine zero calibrator was tested with 12 replicates. The LOD was defined as mean +3SD.

Zero Calibrator

n	12
Mean	0.05
SD	0.117
Mean + 3SD	0.40
LOD =	0.4 µM HCY

Accuracy
Correlation studies were performed by testing 40 serum samples in comparison with an existing commercial Hcy assay method. Linear regression gives a correlation coefficient r2 value of 0.99, slope of 0.94 and y intercept of 1.05.

Precision
Precision studies were conducted according to the NCCLS EP-5 protocol with the following modifications. For within precision, four HCY serum samples containing 7.0, 12.0, 15.6, and 29.0 M HCY were tested with HCY Enzymatic Assay on OLYMPUS AU400 with 20 replicates within one day. Within run imprecisions (CV%) for four levels of Hcy serum samples are 4.5% for 7 M Hcy, 1.87% for 12 M Hcy, 3.04% for 15.6 M Hcy, and 2.4% for 29.0 M Hcy. For inter run precision, four HCY serum samples containing 7.0, 12, 15.6 and 29.0 M HCY were tested with 2 runs per day with triplicates over 5 days. Inter imprecision for three levels of Hcy controls are 5.87% for 7 M Hcy, 4.88% for 12 M Hcy, 5.51% for 15.6 M Hcy, and 2.57% for 29.0 M Hcy.

Linearity
The assay is linear up to 50 µmol/L.

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% devia- tion when tested at the stated concentrations: 40 mg/dL Bilirubin, 1000 mg/dL Triglycerides, 500 mg/dL Hemoglobin, 40 mg/dL Bilirubin Con- jugate, 10 mM Ascorbic Acid, and 100 M** Cystathionine.

** The concentrations tested are about 5-10 times higher than the normal range of serum levels.

References

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Notes on Symbols and Marks

	Consult instructions for use
	Use-by date
LOT	Batch code
REF	Catalouge number
	Caution
	Manufacturer
IVD	*In vitro diagnostic* medical device
	Temperature limit
	Do not re-use
CONT.	The pack contains
	Recycle
	Date of manufacture

ISO 9001, ISO 13485 and ICMED 13485 Certified Company

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