



HOMOCYSTEINE LR liquid reagent

REF 9910135
991S

CE IVD For in vitro medical device

Use

The Liquid Stable (LS) 2-Part Homocysteine Reagent is intended for in vitro quantitative determination of total homocysteine in human serum and plasma. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria. This assay is for professional use only.

Summary

Homocysteine (HCY) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Homocysteine is exported into plasma where it circulates, mostly in its oxidized form, bound to plasma proteins as a protein-HCY mixed disulfide with albumin (protein-SS-HCY).¹⁻⁵ Smaller amounts of reduced homocysteine and the disulfide homocystine (HCY-SS-HCY) are present. Total homocysteine (tHCY) represents the sum of all the HCY species found in serum or plasma (free plus protein bound). Homocysteine is metabolized to either cysteine or methionine. In the vitamin B6 trans-sulphuration pathway, homocysteine is irreversibly catabolized to cysteine. A major part of homocysteine is remethylated to methionine, mainly by the folate and cobalamin-dependent enzyme methionine synthase. Homocysteine accumulates and is excreted into blood when these reactions are impaired.^{3,5} Severely elevated concentrations of total homocysteine are found in subjects with homocystinuria, a rare genetic disorder of the enzymes involved in the metabolism of homocysteine. Patients with homocystinuria exhibit mental retardation, early arteriosclerosis and arterial and venous thromboembolism.^{2,6} Other less severe genetic defects which lead to moderately elevated levels of total homocysteine are also found.⁷⁻⁹

Principle

Bound or dimerised homocysteine (oxidised form) is reduced to free homocysteine, which then reacts with serine catalysed by cystathionine beta-synthase (CBS) to form cystathionine. Cystathionine in turn is broken down by cystathionine beta-lyase (CBL) to form homocysteine, pyruvate and ammonia. Pyruvate is then converted by lactate dehydrogenase (LDH) to lactate with nicotinamide adenine dinucleotide (NADH) as coenzyme. The rate of NADH conversion to NAD⁺ is directly proportional to the concentration of homocysteine ($\Delta A_{340 \text{ nm}}$).

REAGENTS

R 1a Ready-to-use	NADH (0.47 mM), LDH (38 KU/L), Serine (0.76 mM), Trizma Base 1-10%, Trizma Hydrochloride 1-10%, Sodium Azide < 1%. Reductant (TCEP:2.9mM)
R 2 Ready-to-use	Cycling Enzymes CBS (0.748 KU/L) and CBL (16.4 KU/L) Sodium Azide < 1%.
CAL 0 (Blue Cap) Ready-to-use	Aqueous homocysteine blank (0 $\mu\text{mol/L}$).
CAL 1 (Red Cap) Ready-to-use	Aqueous homocysteine solution (28 $\mu\text{mol/L}$).

Storage and stability

- Store the kit at 2-8°C and use until the expiry date on the labels. Do not use expired reagents.

Precaution in Use

The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998). However the reagent should be handled with caution, according to good laboratory practice. Caution: the reagents contain Sodium Azide (0.095%) as preservative. Avoid swallowing and contact with skin, eyes and mucous membranes.

Waste Management

Please refer to the local legal requirements.

Specimen Collection and Preparation

Serum (collected in serum or serum separator tubes) and plasma (collected in potassium EDTA or lithium heparin tubes) may be used for the measurement of homocysteine.

However, it is not recommended to use individual patient results from serum, heparinized plasma and EDTA plasma interchangeably.

To minimize increases in homocysteine concentration from synthesis by red blood cells, process specimens as follows:

Place all specimens (serum and plasma) on ice after collection and prior to processing. Serum may clot more slowly and the volume may be reduced.

All specimens may be kept on ice for up to 6 hours prior to separation by centrifugation.

Separate red blood cells from serum or plasma by centrifugation and transfer to a sample cup or other clean container.

Note: Specimens not placed on ice immediately may exhibit a 10-20% increase in homocysteine concentration.

If the assay will be performed within 2 weeks after collection, the specimen should be stored at 2-8°C. If the testing will be delayed more than 2 weeks, the specimen should be stored frozen at -20°C or colder. Specimens have been shown to be stable at -20°C for 8 months.

Inspect all samples (specimens, calibrators and controls) for bubbles. Remove bubbles prior to analysis.

For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. Avoid the use of severely lipemic specimens.

Mix specimens thoroughly after thawing by low speed vortexing or by gentle inversion to ensure consistency in results. Avoid repeated freezing and thawing. Specimens showing particulate matter, erythrocytes, or turbidity should be centrifuged before testing.

Quality controls

It's necessary, every time the kit is used, to make the quality controls and to check that values obtained are within the acceptance range provided in the insert. Each laboratory should establish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

Reference Values

The reference range should be determined by each laboratory to confirm the characteristics of the population being tested. As a point of reference the following data may be used until the laboratory has analysed a sufficient number of specimens to determine its own reference range. The HCY concentration in plasma or serum of healthy individuals varies with age, gender, geographical area and genetic factors. Scientific literature reports reference values for adult male and females between 5 and 15 $\mu\text{mol/L}$, men having higher values than women, and postmenopausal women having higher homocysteine values than premenopausal women. HCY values will normally increase with age, giving a reference range among an elderly population (> 60 years) of 5-20 $\mu\text{mol/L}$. In countries with folic acid fortification programmes, reduced levels of HCY may be observed.

ANALYTICAL PERFORMANCES

Linearity

Reaction is linear up to a concentration of 50 $\mu\text{mol/L}$.

Analytical sensitivity

The test sensitivity in terms of detection limit is: 0 $\mu\text{mol/L}$.

Correlation

A study based comparing this method(y) with a similar method(x) on 94 samples has given a correlating factor

$$r = 1.0$$

$$y = 0.222 x + 0.985$$

"Intra-Assay" precision (within-Run)

A study was performed with guidance from the CLSI (formerly NCCLS) Document EP5-A2.²⁸ Three HCY controls and three human plasma panels were assayed using two lots of reagents, in replicates of two, at two separate times per day for 20 days on one instrument (n=80). A calibration curve was generated at the start of the study and was used throughout. Results (rounded to 1 decimal place) are summarised below

Sample	Reagent Lot	Mean ($\mu\text{mol/L}$)	Within Run CV%	Total CV%
Panel 1	1	7.0	1.9	3.3
	2	7.0	2.2	4.4
Panel 2	1	36.0	1.3	2.5
	2	35.5	1.1	2.3
Panel 3	1	48.3	1.1	2.0
	2	47.7	1.0	2.2
Low Control	1	6.3	2.6	4.4
	2	6.3	2.1	4.1
Medium Control	1	12.3	1.5	3.0
	2	12.2	1.3	3.2
High Control	1	25.5	1.5	2.5
	2	25.3	1.6	2.9

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Interferences

No interference was observed by the presence of:

Bilirubin \leq 10 mg/dl.

Triglycerides \leq 500 mg/dl.

Hemoglobin \leq 500 mg/dl.

A list of drugs and other interfering substances with lipase determination has been reported by Young et al.²³.

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Simbols

CE CE Mark (requirement of 98/79 regulation)

IVD in vitro medical device

LOT Batch Code

Use by

Storage temperature limits

Read instruction for use

Gesam Production srl

HOMOCYSTEINE LR
MOD.7.3.5 Rev. 0 del 2006-03

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The following instructions may be used as guidance for preparation of an instrument protocol for use with the Gesan 2-reagent Enzymatic Homocysteine assay on a suitable clinical chemistry analyzer.

Materials required but not provided

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

Analytical steps

The following guidance may be used for preparing the instrument protocol.

