HOMOCYSTEINE LR liquid reagent

REF 9910135 R1 1x30 R2 1x5
991S R1 1x15 R2 1x2,5

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 catabolised 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 concentrated 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 (Δ A340 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 µmol/L).
CAL 1 (Red Cap) **Ready-to-use**	Aqueous homocysteine solution (28 µmol/L).

Storage and stability

- Store the kit at 2-8°C and use until the expiry date on the labels. Do not use exired 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 laboratori should estabilish 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** µmol/L, men having higher values than women, and post menopausal woman having higher homocysteine values than pre-menopausal women. HCY values will normally increase with age, giving a reference range among an elderly population (> 60 years) of 5-20 µmol/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 µmol/L.

Analytical sensitivity

The test sensitivity in terms of detection limit is: 0 µmol/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 (formally 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 (µmol/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 ≤ 10 mg/dl.
Triglycerides ≤ 500 mg/dl.
Hemoglobin ≤ 500 mg/dl.
A list of drugs and other interfering substances with lipase determination has been reported by Young et. al[2,3].

BIBLIOGRAPHY

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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
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