

Liquid Stable (LS) 2-Part HOMOCYSTEINE REAGENT

REF FHRK100



For professional use only
On the **Roche MODULAR ANALYTICS <P>**



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ENGLISH:**INTENDED 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 AND EXPLANATION OF TEST

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 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

Epidemiological studies have investigated the relationship between elevated homocysteine levels and cardiovascular disease (CVD). A meta-analysis of 27 of these studies, including more than 4000 patients, estimated that a 5 µmol/L increase in total homocysteine was associated with an odds ratio for coronary artery disease (CAD) of 1.6 (95% confidence interval [CI], 1.4 to 1.7 for men and 1.8 (95% CI 1.3 to 1.9) for women; the odds ratio for cerebrovascular disease was 1.5 (95% CI 1.3 to 1.9). The risk associated with a 5 µmol/L increase in total homocysteine was the same as that associated with 0.5 mmol/L (20 mg/dL) increase in cholesterol. Peripheral arterial disease also showed a strong association.¹⁰

Hyperhomocysteinemia, elevated levels of homocysteine, can be associated with an increased risk of CVD. There have also been many published reports of prospective studies on the relationship between hyperhomocysteinemia and risk of CVD in men and women who were initially healthy. End points were based on a cardiovascular event such as acute myocardial infarction, stroke, CAD, or mortality. The results of eleven of these nested case-control studies reviewed by Cattaneo¹¹ were equivocal where five of the studies support the association with risk and six do not. More recently homocysteine levels were determined in a prospective study of post-menopausal women who participated in the Women's Health Study. Specimens from 122 women, who subsequently developed cardiovascular events, were tested for homocysteine and compared to a control group of 244 women who were matched for age and smoking status. The women in the control group remained free of disease during the three year follow-up period. The results demonstrated that post-menopausal women who developed cardiovascular events had significantly higher baseline homocysteine levels. Those with levels in the highest quartile had a two-fold increase in risk of any cardiovascular event. Elevated baseline homocysteine levels were shown to be an independent risk factor.¹² Also, homocysteine levels were determined in 1933 elderly men and women for the Framingham Heart Study cohort and demonstrated that elevated levels of homocysteine are independently associated with increased rates of all-cause and CVD mortality.¹³

Patients with chronic renal disease experience an excess morbidity and mortality due to arteriosclerotic CVD. Elevated concentration of homocysteine is a frequently observed finding in the blood of these patients. Although such patients lack some of the vitamins involved in the metabolism of homocysteine, the elevated HCY levels are mainly due to impaired HCY removal from the blood by the kidneys.^{14,15}

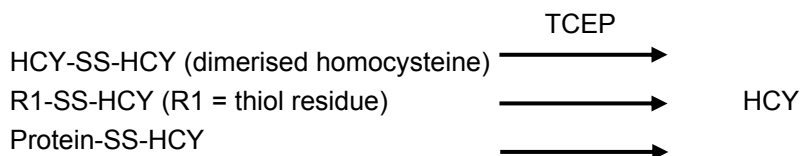
Recent evidence has also implicated elevated blood levels of homocysteine in miscarriages and birth defects.¹⁶

Drugs such as methotrexate, carbamazepine, phenytoin, nitrous oxide, and 6-azauridine triacetate interfere with HCY metabolism and may give elevated levels of HCY.¹⁷

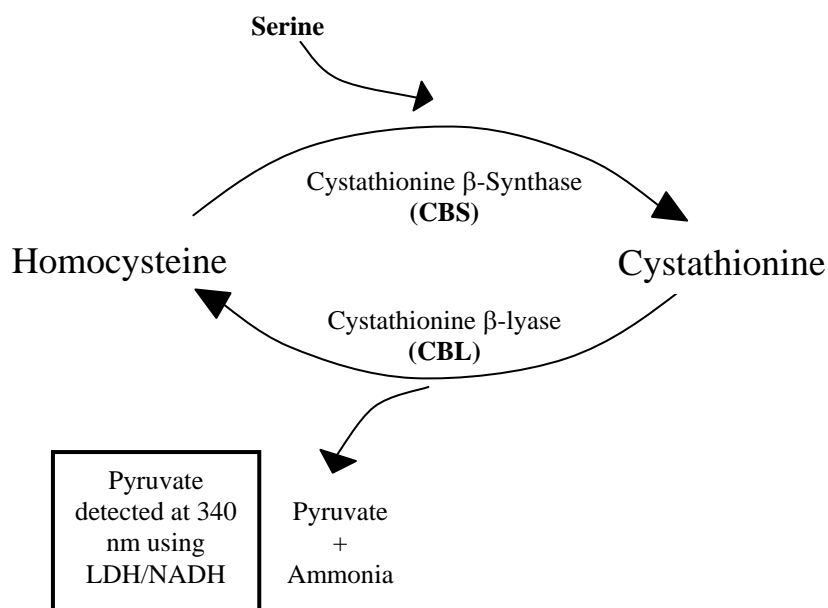
PRINCIPLE OF THE ASSAY

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 ($\Delta A_{340 \text{ nm}}$).

Reduction: Dimerised homocysteine, mixed disulfide, and protein-bound forms of HCY in the sample are reduced to form free HCY by the use of tris [2-carboxyethyl] phosphine (TCEP).



Enzymatic Conversion: Free HCY is converted to cystathionine by the use of cystathionine beta-synthase and excess serine. The cystathionine is then broken down to homocysteine, pyruvate and ammonia. Pyruvate is converted to lactate via lactate dehydrogenase with NADH as coenzyme. The rate of NADH conversion to NAD^+ ($\Delta A_{340 \text{ nm}}$) is directly proportional to the concentration of homocysteine.



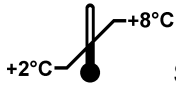
KIT COMPONENTS

REAG 1	Colourless odourless liquid	1 x 30 mL in Amber Vial	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:2.9 mM) Ready-to-use	Xn
REAG 2	Pale yellow odourless liquid	1 x 5.0 mL in Amber Vial	Cycling Enzymes CBS (0.748 KU/L) and CBL (16.4 KU/L) Sodium Azide < 1%. Ready-to-use	Xn
CAL	Colourless odourless liquid	1 x 3.0 mL in Opaque Vial (Blue Cap)	Aqueous homocysteine blank (0 µmol/L). Ready-to-use	
CAL	Colourless odourless liquid	1 x 3.0 mL in Opaque Vial (Red Cap)	Aqueous homocysteine solution (28 µmol/L). Ready-to-use	

The calibrators are prepared gravimetrically and are traceable to NIST SRM 1955, confirmed by a designated measurement procedure (HPLC). The values assigned are printed on the labels (0 µmol/L and 28 µmol/L).

STORAGE OF REAGENTS

Handling and Procedural Notes

- 
- Store kit components at 2-8°C and use until the expiry date on the labels. Do not use expired reagents.
 - Do not mix different reagent kit lot numbers.
 - DO NOT FREEZE REAGENTS.**
 - Do not expose Reagent 1 and Reagent 2 to light during on-board use.
 - Avoid contamination of reagents. Use a new disposable pipette tip for each reagent or sample manipulation.
 - Onboard instrument storage. The reagents can be stored for 30 days on-board the Roche MODULAR ANALYTICS <P>.

Indications of Deterioration

The reagents should be clear of particulate material. They should be discarded if they become turbid.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only

Safety Precautions

1. Adhere strictly to the instructions in this leaflet, particularly for handling and storage conditions.
2. Reagent 1 and Reagent 2 contain sodium azide which can react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large quantities of water to prevent azide build-up.
3. Material safety data sheets for all hazardous components contained in this kit are available upon request from Axis-Shield Diagnostics Ltd.



R22: Harmful if swallowed.

R32: Contact with acids liberates very toxic gas.

S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.

S29/35: Do not empty into drains; dispose of this material and its container in a safe way.

S46: If swallowed, seek medical advice immediately and show this container or label.

SPECIMEN COLLECTION AND HANDLING

1. 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.¹⁷
- All specimens may be kept on ice for up to 6 hours prior to separation by centrifugation.¹⁷
- 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.¹⁸

2. 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.^{17,19}
3. It is the responsibility of the operator to verify the correct specimen type(s) is (are) used in the Liquid Stable (LS) 2-Part Homocysteine Reagent.
4. Inspect all samples (specimens, calibrators and controls) for bubbles. Remove bubbles prior to analysis.
5. For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. Avoid the use of severely lipemic specimens.
6. 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.
7. On-board instrument storage. The samples can be stored for 3 hours on-board the Roche MODULAR ANALYTICS <P>.

RESULTS

Results are printed out by the Roche MODULAR ANALYTICS <P> in $\mu\text{mol/L}$.

QUALITY CONTROL PROCEDURES

Ensure that adequate maintenance and calibration is performed according to the manufacturer's instructions.

Assayed control materials with values for homocysteine in both the normal and abnormal ranges should be tested to validate reagent performance. Users should ensure that they are fully acquainted with the instructions for the assay, particularly the Warnings and Precautions section, and the Handling and Procedural Notes. Users should demonstrate that they obtain performance specifications for precision and reportable range of test results comparable to those established by the manufacturer before reporting patient test results.

An Axis-Shield Homocysteine Control Kit (FHCY200) containing low, medium and high controls is also available from Axis-Shield for use with the Liquid Stable (LS) 2-Part Homocysteine Reagent.

EXPECTED VALUES

Reference Range: 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 post menopausal woman having higher homocysteine values than pre-menopausal women.^{17,20,21} HCY values will normally increase with age, giving a reference range among an elderly population (> 60 years) of 5-20 $\mu\text{mol/L}$.²² In countries with folic acid fortification programmes, reduced levels of HCY may be observed.^{23,24}

Measurable Range: The measurable range of the Liquid Stable (LS) 2-Part homocysteine assay is 0-50 $\mu\text{mol/L}$. Samples within the range of the assay were diluted according to guidance in the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Document EP6-A²⁹ using Cal 0 $\mu\text{mol/L}$.

LIMITATIONS OF USE

1. The linear range of the Liquid Stable (LS) 2-Part Homocysteine Reagent when run as directed is 50 $\mu\text{mol/L}$.
2. The Reagents should be clear. Discard if turbid.
3. Cystathionine is measured with homocysteine, but in the general population the cystathionine level (0.065 to 0.3 $\mu\text{mol/L}$) has a negligible effect. In very rare cases, end stage renal disease and patients with severe metabolic disturbances, cystathionine levels may rise dramatically and in severe cases cause greater than 20% interference.^{25,26}
4. Hydroxylamine, present in several iron reagents may carryover (reagent probe or reaction cuvette) and cause falsely low results. Routine rinsing procedures are not adequate to eliminate this problem in most cases. Possible solutions would include special washing protocols, changing to an iron assay that used ascorbic acid as reductant or running iron and homocysteine assays on separate instruments.
5. Carbamazepine, methotrexate, phenytoin, nitrous oxide, or 6-azauridine triacetate may affect the homocysteine concentration.¹⁷
6. Samples with raised protein levels show >10% difference compared to results obtained from normal samples and should be avoided.

PERFORMANCE DATA – Roche MODULAR ANALYTICS <P>

Accuracy

A correlation study was performed with plasma specimens from apparently healthy adults. All specimens were analysed using the Liquid Stable (LS) 2-Part Homocysteine Reagent and the Catch Liquid Stable (LS) 2-Part Homocysteine Reagent according to the CLSI (formally NCCLS) document EP9-A2.²⁷ All results are based on 95% confidence Interval. Liquid Stable (LS) 2-Part Homocysteine Reagent specimens ranged from 5.7 to 47.1 µmol/L. The data obtained gave the following statistical values:

Comparison Method	Axis-Shield v Catch
<i>Number of specimens</i>	94
<i>Slope of regression line</i>	0.933
<i>Y-Intercept</i>	-0.175
<i>Correlation coefficient</i>	1.0

Precision

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%
<i>Panel 1</i>	1	6.4	3.3	6.8
	2	6.4	2.7	6.6
<i>Panel 2</i>	1	33.9	1.7	2.8
	2	33.9	2.1	2.9
<i>Panel 3</i>	1	45.7	1.1	2.0
	2	45.6	1.0	2.0
<i>Low Control</i>	1	6.0	4.9	5.7
	2	6.2	4.0	5.0
<i>Medium Control</i>	1	11.8	1.9	3.1
	2	11.9	1.9	3.2
<i>High Control</i>	1	24.3	1.2	1.9
	2	24.5	1.0	2.4

Dilution Linearity

The dilution linearity of the Liquid Stable (LS) 2-Part Homocysteine Reagent according to guidance in the CLSI (formally NCCLS) Document EP6-A,²⁹ gives mean recovery across the dilutions for each of the samples of 100% \pm 4% across the range of the assay (6 - 50 μ mol/L) on the Roche MODULAR ANALYTICS <P>.

Limit of Detection

The limit of detection (LOD) of the Liquid Stable (LS) 2-Part Homocysteine Reagent according CLSI (formally NCCLS) Document EP17-A³⁰ was found to be \leq 1.0 μ mol/L.

Analytical Specificity

The specificity of the Liquid Stable (LS) 2-Part Homocysteine Reagent was assessed according to guidance in the CLSI Document EP7-A2³¹ for the interfering substances listed in the table below:

<i>Interfering Substance</i>	<i>Interfering Substance Concentration</i>	<i>% Interference</i>
<i>Bilirubin</i>	20 mg/dL	\leq \pm 10
<i>Haemoglobin</i>	500 mg/dL	\leq \pm 10
<i>Red Blood Cell</i>	0.4%	\leq \pm 10
<i>Triglyceride</i>	500 mg/dL	\leq \pm 10
<i>Glutathione</i>	1000 μ mol/L	\leq \pm 10
<i>Methionine</i>	800 μ mol/L	\leq \pm 10
<i>Cysteine</i>	200 μ mol/L	\leq \pm 10
<i>Pyruvate</i>	1250 μ mol/L	\leq \pm 10

None of these substances interfered significantly in the assay.

Refer to to page 11 of this pack leaflet (ref 17) for possible interferences caused by drugs, disease or preanalytical variables.

Carryover

Carryover studies on the Roche MODULAR ANALYTICS <P> show that carryover is less than the limit of detection of the assay.

Onboard Reagent Stability

The reagents are stable onboard the Roche MODULAR ANALYTICS <P> for 30 days.

Calibration Stability

The calibration curve on the Roche MODULAR ANALYTICS <P> is stable for 30 days.

% Recovery

The Liquid Stable (LS) 2-Part Homocysteine Reagent gives a % recovery of 100 \pm 10% for the mean of each specimen of each of the following tube types (lithium heparin, serum and serum separator tubes) when compared with the corresponding potassium EDTA specimen mean value on the Roche MODULAR ANALYTICS <P>

ASSAY PROTOCOL – Roche MODULAR ANALYTICS <P>

Roche MODULAR ANALYTICS <P> Procedure Parameters:

Test: HCY*	Type: Ser/PI
ANALYZE	
Assay time/Point	[2 Point End]/[10]/[19] [34] [0] [0]
Wave (2nd/Primary)	[376]/[340]
S. Vol (Normal)	[16.5]
Reagent (R1) T1	[250] [0] [000000]
Reagent (R2) T2	[0] [0] [000000]
Reagent (R3) T3	[25] [0] [000000]
Abs. Limit	[32000] [Decrease] 2 Tests
Prozone Limit	[-32000] [0] [Lower]
Cell Detergent	[Detergent 1]
CALIB	
Calibration Type	[Linear]
Point	[2]
Span Point	[2]
Weight	[0]
Auto Calibration	
2 Point	[168]
SD Limit	[100]
Duplicate Limit	[10%] [32000 Abs]
Sensitivity Limit	[-99999] [99999]
S1 Abs limit	[-32000] [32000]
RANGE	
Application Code*	[] Unit [µmol/L]
Control Interval*	[]
Instrument Factor	(Y=aX+b) a=[1.0] b=[0.0]
Technical Limit	[1.0] [50.0]
Repeat Limit*	[-99999] [99999]
OTHERS	
<Standard>	(1) (2)
Calibration Code*	[] []
Concentration**	[0.00] [**]
Position*	[] []
Sample Volume	[16.5] [16.5]

*User Defined

**Enter Values on Calibrator Vials

Ensure that the assay parameters exactly match those listed above.

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IVD

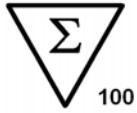
In Vitro Diagnostic Medical Device

REF

Product code

LOT

Lot number



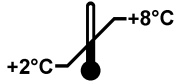
100 tests



Consult Instructions For Use



Use by



Store at 2-8°C



Manufactured by



Store in the dark

REAG 1

Reagent 1, 2

CAL

Calibrator 0 $\mu\text{mol/L}$, Calibrator 28 $\mu\text{mol/L}$



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