HCYS

cobas®

Homocysteine Enzymatic Assay

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05385415 190	Homocysteine Enzymatic Assay 100 tests	System-ID 07 7487 1	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
05385504 190	HCYS Calibrator Kit (2 x 3 mL)	Code 590 (HCYCA)	
05142423 190	HCYS Control Kit Control 1 (2 x 3 mL)	Code 254 (HCYC1)	
	HCYS Control Kit Control 2 (2 x 3 mL)	Code 255 (HCYC2)	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

HCYS: ACN 778

For **cobas c** 502 analyzer:

HCYS: ACN 8778

Intended use

In vitro test for the quantitative determination of total L-homocysteine in human serum and plasma on Roche/Hitachi **cobas c** systems. The assay can assist in the diagnosis of patients suspected of having hyperhomocysteinemia or homocystinuria.

Summary[1,2,3]

Homocysteine (Hcy) is a thiol-containing 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.

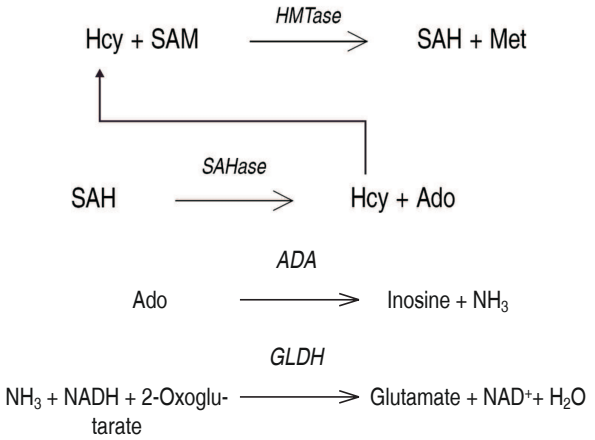
Elevated levels of tHcy has emerged as an important risk factor in the assessment of cardiovascular disease.[1,2,3] Excess Hcy in the blood stream may cause injuries to arterial vessels due to its irritant nature, and result in inflammation and plaque formation, which may eventually cause blockage of blood flow to the heart.

Elevated tHcy levels are caused by four major factors, including:

1. genetic deficiencies in enzymes involved in Hcy metabolism such as cystathionine beta-synthase (CBS), methionine synthase (MS), and methylenetetrahydrofolate reductase (MTHFR);
2. nutritional deficiency in B vitamins such as B_6, B_{12} and folate;
3. renal failure for effective amino acid clearance; and
4. drug interactions, such as with nitric oxide, methotrexate and phenytoin that interfere with Hcy metabolism. Elevated levels of tHcy are also linked with Alzheimer's disease[4], neuropsychiatric diseases[5] and Osteoporosis.[6] Guidelines for tHcy determination in clinical laboratories have been established.[7,8]

Test principle

Homocysteine Enzymatic Assay is based on a novel enzyme cycling assay principle that assesses the co-substrate conversion product instead of assessing co-substrate or Hcy conversion products of Hcy. In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM), to form methionine (Met) and S-adenosylhomocysteine (SAH), catalyzed by a Hcy S-methyltransferase. SAH is assessed by coupled enzyme reactions where SAH is hydrolyzed into adenosine (Ado) and Hcy by SAH hydrolase, and Hcy is cycled into the Hcy conversion reaction to form a reaction cycle that amplifies the detection signal. 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^+ ($\Delta A_{340\ nm}$).



Reagents - working solutions

R1 **NADH reagent**
S-adenosylmethionine 0.1 mmol/L, TCEP* > 0.5 mmol/L, 2-oxoglutarate < 5.0 mmol/L, NADH > 0.2 mmol/L, buffer, pH 9.1 (25 °C), preservative, stabilizer

R2 **Enzyme reagent**
Homocysteine S-methyltransferase (HMTase) 5.0 kU/L, glutamate dehydrogenase (GLDH) 10 kU/L, casein (bovine) ≤ 0.2 %, buffer, pH 7.2 (25 °C), preservative, detergent

R3 **Start reagent**
Adenosine deaminase (bovine) 5.0 kU/L, S-adenosyl-homocysteine hydrolase (SAHase) 3.0 kU/L, casein (bovine) ≤ 0.2 %, buffer, pH 7.2 (25 °C), preservative, stabilizer

*Tris(2-carboxyethyl)phophine

R1 is in postion A, R2 is in postition B and R3 is in postion C.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

HCYS

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	4 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.

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Homocysteine Enzymatic Assay

On-board in use and refrigerated on the analyzer: 12 weeks

Do not freeze.

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin, K_2-EDTA and K_3-EDTA plasma.

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 ice and centrifuged within an hour. Hemolysed or turbid specimens or severely lipemic specimens are not recommended for the Hcy assay.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Stability:[8,9,10]	4 days at 15-25 °C
	4 weeks at 2-8 °C
	10 months at -20 °C

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 36-57		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	µmol/L		
Reagent pipetting		Diluent (H_2O)	
R1	176 µL	–	
R2	28 µL	–	
R3	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	14 µL	–	–
Decreased	14 µL	30 µL	120 µL
Increased	14 µL	–	–

cobas c 501/502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 51-70		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Units	µmol/L		
Reagent pipetting		Diluent (H_2O)	
R1	176 µL	–	
R2	28 µL	–	
R3	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	14 µL	–	–
Decreased	14 µL	30 µL	120 µL
Increased	14 µL	–	–

Calibration

Calibrators	S1-5: HCYS Calibrator Kit
	Multiply the lot-specific HCYS Calibrator Kit calibrator value by the factors below to determine the standard concentrations for the 5-point calibration curve:
	S1: 0.050 S4: 0.500
	S2: 0.100 S5: 1.00
	S3:0.250
Calibration mode	RCM
Calibration frequency	Full calibration • every 7 days • after reagent lot change • and as required following quality control procedures

Traceability: This method has been standardized against NIST SRM 1955 reference material.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

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 defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Limitations - interference

Criterion: Recovery within ± 10 % of initial value for analyte concentrations > 15 µmol/L or ± 1.5 µmol/L for analyte concentrations ≤ 15 µmol/L.

Icterus:[11] No significant interference up to an I index of 20 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 342 µmol/L (20 mg/dL)).

Hemolysis:[11] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62 µmol/L (100 mg/dL)).

Lipemia (Intralipid):[11] No significant interference up to an L index of 250. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12,13]

Exceptions: 0.5 mmol/L Glutathione, 100 µmol/L Cystathionine, 0.5 mmol/L Pyruvate.

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.[7,10]

S-Adenosylhomocysteine (SAH) will cause a significant positive interference. However, SAH is not detectable at sub-nmol/L concentrations in normal plasma, and should not cause concern.[14]

Addition of 3-deazaadenosine to inhibit Hcy production in red cells has been suggested. However, the Homocysteine Enzymatic Assay can not use samples containing 3-deazaadenosine since it inhibits one of the key enzymes used in the assay.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[15]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

3-50 µmol/L

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

LoB = 3 µmol/L

LoD = 3 µmol/L

LoQ = 5.5 µmol/L

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 30 %. It has been determined using low concentration homocysteine samples.

Expected values

In most of the U.S. clinical laboratories, 15 µmol/L is used as the cut-off value for normal levels of Hcy in adults.

In European laboratories, 12 µmol/L is used as the cut-off value for normal levels of Hcy in adults:[8]

Age, pregnancy, and renal function are important. The intake of folic acid as either supplements or through fortification of foods must also be considered:

Group	Folate supplemented	Nonsupplemented
Fasting/basal tHcy, µmol/L		
Pregnancy	8	10
Children < 15 years	8	10
Adults 15-65 years	12	15
Elderly > 65 years	16	20

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements with repeatability (n = 21) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
Homocysteine Control 1	12.2	0.2	1.5
Homocysteine Control 2	39.1	0.7	1.8
Human serum 1	8.26	0.16	2.0
Human serum 2	13.1	0.2	1.8
Human serum 3	30.0	0.4	1.4
Human serum 4	44.4	0.9	2.0

Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
Homocysteine Control 1	12.2	0.3	2.1
Homocysteine Control 2	39.1	0.8	2.0
Human serum 1	8.26	0.19	2.3
Human serum 2	13.1	0.3	2.1
Human serum 3	30.0	0.5	1.8
Human serum 4	44.4	1.0	2.2

Method comparison

Hcy values for human serum samples obtained on the **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a COBAS INTEGRA 400 analyzer (x).

Sample size (n) = 56

Passing/Bablok[16]	Linear regression
y = 0.962x + 0.248 µmol/L	y = 0.993x - 0.175 µmol/L
τ = 0.971	r = 0.999

The sample concentrations were between 3.03 and 47.2 µmol/L.

References

1 Eikelboom JW, Lonn E, Genest J Jr, et al. Homocyst(e)ine and cardiovascular disease: A critical review of the epidemiologic evidence. Ann Intern Med 1999;131(5):363-375.

2 Scott J, Weir D. Homocysteine and cardiovascular disease. Q J Med 1996;89(8):561-563.

3 Nygard O, Nordrehaug JE, Refsum H, et al. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997;337(4):230-236.

4 Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346(7):476-483.

5 Stanger O, Fowler B, Piertzik K, et al. Homocysteine, folate and vitamin B12 in neuropsychiatric diseases: review and treatment recommendations. Expert Rev Neurother 2009;9(9):1393-1412.

6 McLean RR, Jacques PF, Selhub J, et al. Homocysteine as a predictive factor for hip fracture in older persons. N Engl J Med 2004;350(20):2042-2049.

7 Refsum H. Total Homocysteine: Guidelines for Determination in the Clinical Laboratory. Clin Lab News 2002 May;12-14 (www.aacc.org).

8 Refsum H, Smith AD, Ueland PM, et al. Facts and Recommendations about Total Homocysteine Determinations: An Expert Opinion. Clin Chem 2004;50(1):3-32.

9 Fiskerstrand T, Refsum H, Kvalheim G, et al. Homocysteine and other thiols in plasma and urine: Automated determination and sample stability. Clin Chem 1993 Feb;39(2):263-271.

10 Rasmussen K and Moller J. Total homocysteine measurement in clinical practice. Ann Clin Biochem 2000;37:627-648.

11 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

12 Breuer J. Report on the Symposium “Drug effects in Clinical Chemistry Methods”. Eur J Clin Chem Clin Biochem 1996;34:385-386.

13 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

14 Loehrer FM, Angst CP, Brunner FP, et al. Evidence for disturbed S-adenosylmethionine : S-adenosylhomocysteine ratio in patients with end-stage renal failure: a cause for disturbed methylation reactions? Nephrol Dial Transplant 1998;13(3):656-661.

15 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

16 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing

Significant additions or changes are indicated by a change bar in the margin.

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