0108057826190c503V6.0

HCYS

cobas®

Homocysteine Enzymatic Assay

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057826190	Homocysteine Enzymatic Assay (**cobas c** pack 1), 100 tests	System-ID 2070 011	**cobas c** 303, **cobas c** 503
	Homocysteine Enzymatic Assay (**cobas c** pack 2), 100 tests	System-ID 2070 012	
Materials required (but not provided):			
05385504190	HCYS Calibrator Kit (2 x 3 mL)	Code 20590	
05142423190	HCYS Control Kit Control 1 (2 x 3 mL)	Code 20254	
	HCYS Control Kit Control 2 (2 x 3 mL)	Code 20255	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

HCYS: ACN 20700

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.

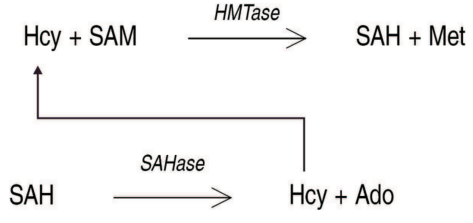
Elevated levels of tHcy has emerged as an important risk factor in the assessment of cardiovascular disease.[1,2,3] Excess Hcy in the blood stream may cause injuries to arterial vessels due to its irritant nature, and result in inflammation and plaque formation, which may eventually cause blockage of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

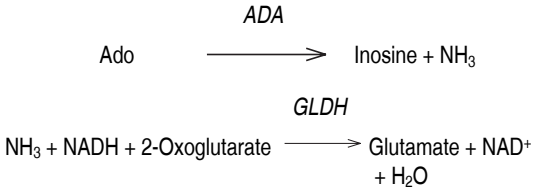
Elevated tHcy levels are caused by four major factors, including:

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. 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)phosphine

Cat. No. 08057826190 consists of 2 **cobas c** packs: 1 x R1 + R2 and 1 x R3. R1 is in position B and R2 is in position C of **cobas c** pack 1. R3 is in position C of **cobas c** pack 2.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

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

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.

See the limitations and interferences section for details about possible sample interferences.

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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	132 µL	–	
R2	21 µL	–	
R3	15 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	10.5 µL	–	–
Decreased	10.5 µL	25.0 µL	100 µL
Increased	10.5 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1-5: HCYS Calibrator Kit
Calibration mode	Non-linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - every 7 days - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 4 weeks.

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

cobas c systems automatically calculate the analyte concentration of each sample in the unit µmol/L.

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 or 20 mg/dL).

Hemolysis:[11] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62 µmol/L or 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 interference with Hcy metabolism.[7,10]

S-Adenosylhomocysteine (SAH) will cause a significant positive interference. However, SAH is only 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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet for information. For further instructions refer to the operator's manual.

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, Limit of Detection and Limit of Quantitation

Limit of Blank	= 3 µmol/L
Limit of Detection	= 3 µmol/L
Limit of Quantitation	= 5.5 µmol/L

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 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 (fasting/basal tHcy, µmol/L)	Folate supplemented	Nonsupplemented
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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean* µmol/L	*SD* µmol/L	*CV* %
Homocysteine Control 1	13.2	0.149	1.1
Homocysteine Control 2	37.2	0.462	1.2
Human serum 1	9.52	0.135	1.4
Human serum 2	12.1	0.195	1.6
Human serum 3	15.0	0.297	2.0
Human serum 4	26.2	0.433	1.7
Human serum 5	45.3	0.704	1.6
Intermediate precision	*Mean* µmol/L	*SD* µmol/L	*CV* %
Homocysteine Control 1	13.2	0.374	2.8
Homocysteine Control 2	37.3	0.763	2.0
Human serum 1	9.55	0.330	3.5
Human serum 2	12.1	0.437	3.6
Human serum 3	15.4	0.447	2.9
Human serum 4	26.2	0.653	2.5
Human serum 5	45.3	0.945	2.1

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Method comparison

Hcy values for human serum samples obtained on the **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 74

Passing/Bablok[16]	Linear regression
y = 0.984x + 0.122 µmol/L	y = 0.979x + 0.215 µmol/L
τ = 0.979	r = 0.999

The sample concentrations were between 4.11 and 48.4 µmol/L.

Hcy values for human serum samples obtained on the **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 82

Passing/Bablok[16]	Linear regression
y = 1.014x - 0.407 µmol/L	y = 1.010x - 0.319 µmol/L
τ = 0.951	r = 0.997

The sample concentrations were between 3.53 and 48.7 µmol/L.

References

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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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

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