HCYS

cobas®

Homocysteine Enzymatic Assay

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
06542921190	06542921500	Homocysteine Enzymatic Assay (200 tests)	System-ID 03 7487 1	**cobas c** 701/702
			System-ID 04 7487 1	

Materials required (but not provided):

05385504190	HCYS Calibrator Kit (2 x 3 mL)	Code 590	
05142423190	HCYS Control Kit Control 1 (2 x 3 mL)	Code 254	
	HCYS Control Kit Control 2 (2 x 3 mL)	Code 255	
05172152190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3	

English

System information

HCYS: ACN 8778

Intended use

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

Summary

Homocysteine (Hcy) measurements, performed with this assay in human serum and plasma, are used as an aid in the diagnosis of patients suspected of having hyperhomocysteinemia or homocystinuria.

Homocysteine (Hcy) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Hcy is metabolized by one of two pathways: transsulfuration and remethylation. B-vitamins and folate are needed as substrates in the homocysteine metabolism. Elevation of plasma Hcy levels is called hyperhomocysteinemia. Total homocysteine (tHcy) represents the sum of all forms of Hcy including oxidized, protein-bound, thiolactone and free forms. Hyperhomocysteinemia can be defined in relation to tHcy levels as either moderate, intermediate, or severe.[1,2] Elevations in Hcy levels can be due to:

1. genetic deficiencies in enzymes involved in Hcy metabolism such as cystathionine beta-synthase (CBS), methionine synthase, and methylenetetrahydrofolate reductase;
2. nutritional deficiency in B-vitamins (B6, B12 and folate), levels of which correlate inversely with Hcy levels;
3. renal failure due to defective clearance of Hcy from the kidney;
4. other causes including thyroid dysfunction, cancer, psoriasis, and diabetes as well as various drugs, alcohol, tobacco, coffee, older age and menopause.[2,3]

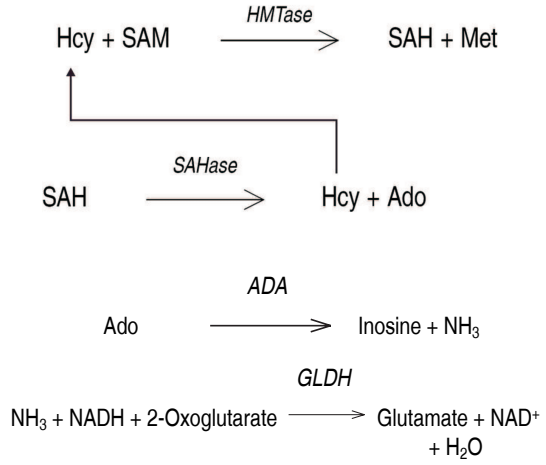
Severe hyperhomocysteinemia occurs in homocystinuria, an innate metabolic disorder characterized by deficiencies in cystathionine β-synthase (CBS), one of the enzymes involved in the metabolism of Hcy to cysteine.[4] For diagnosis of CBS deficiency, measuring tHcy levels is recommended. The diagnosis is very likely if elevated tHcy is accompanied by high or borderline-high plasma methionine-concentrations, and further supported if sensitive methods demonstrate low plasma-cystathionine concentrations with an increased methionine-to-cystathionine ratio.[5,6] Hyperhomocysteinemia is also the hallmark of remethylation inherited disorders. Plasma tHcy is recommended as the first biochemical parameter to assess when a remethylation disorder is suspected. Individuals with remethylation disorders typically have elevated tHcy concentrations but – in contrast to those with CBS deficiency – methionine levels are usually normal or decreased.[6,7] Measurement of tHcy is also recommended to rule out CBS deficiency in the differential diagnosis of inherited methylation disorders such as methionine adenosyltransferase I/III deficiency and glycine N-methyltransferase deficiency. In this case, a low or only slightly increased tHcy level can help exclude CBS deficiency.[8] Elevated levels of tHcy have been found to be associated with numerous pathological conditions, including cardiovascular disease (CVD), stroke, Alzheimer's disease, eye diseases, chronic kidney disease, bone tissue damages, gastrointestinal disorders, cancer, congenital defects, and pregnancy complications.[2,3,9,10,11,12,13,14,15,16,17,18,19,20,21]

1. Hcy has been considered as a marker for CVD risk by clinical societies, further investigations are required in order to evaluate if it is also to be seen as a true causal risk factor.[9,10,11,22,23]

Guidelines for tHcy determination in clinical laboratories have been established.[24,25]

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)phosphine

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Homocysteine Enzymatic Assay

Cat. No. 06542921190 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
On-board on the reagent manager:	1 hour

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.

Centrifuge samples containing precipitates before performing the assay.

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

Stability:	4 days at 15-25 °C[25,26]
	4 weeks at 2-8 °C[27]
	10 months at -20 °C (± 5°C)[27]

Freeze only once.

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 701/702 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 28-38		
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 on-board • after reagent lot change • 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. 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.

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:[28] 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:[28] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62 µmol/L or 100 mg/dL).

Lipemia (Intralipid):[28] 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.[29,30]

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.[24,27]

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.[31]

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.[32]

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, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator's manual.

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, 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 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:[25]

Age, pregnancy, and renal function are important. The intake of folic acid as either supplement 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 on the **cobas c** 701 analyzer:

Repeatability	*Mean*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
Homocysteine Control 1	12.3	0.2	1.3
Homocysteine Control 2	38.9	0.4	1.1
Human serum A	6.15	0.13	2.1
Human serum B	16.9	0.2	1.4
Human serum C	23.3	0.3	1.3
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

Results for intermediate precision were obtained on the **cobas c** 501 analyzer.

The data obained on **cobas c** 501 analyzer(s) are representative for **cobas c** 701 analyzer(s).

Method comparison

Hcy values for human serum samples obtained on the **cobas c** 701 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 62

Passing/Bablok[33]	Linear regression
y = 0.979x + 0.267 µmol/L	y = 0.974x + 0.212 µmol/L
τ = 0.974	r = 0.997

The sample concentrations were between 3.65 and 49.0 µmol/L.

References

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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 navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

HCYS

Homocysteine Enzymatic Assay

cobas®

CE 0123

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