HCYS

Homocysteine Enzymatic Assay

cobas®

Substrates

Order information

REF	CONTENT		Analyzers on which **cobas c** pack can be used
05385415 190	Homocysteine Enzymatic Assay (100 tests)	System-ID 07 7487 1	COBAS INTEGRA 400 plus COBAS INTEGRA 800

Materials required (but not provided):

05385504 190	HCYS Calibrator Kit (2 x 3 mL)	System-ID 07 7493 6	
05142423 190	HCYS Control Kit Control 1 (2 x 3 mL) HCYS Control Kit Control 2 (2 x 3 mL)	System-ID 07 7490 1 System-ID 07 7492 8	
20756350 322	NaCl Diluent 9 % (6 x 22 mL)	System-ID 07 5635 0	

English

System information

Test HCYS, test ID 0-006

Intended use

In vitro test for the quantitative determination of total L-homocysteine in human serum and plasma on COBAS INTEGRA 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.

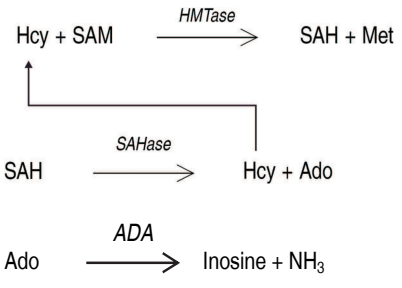
Elevated levels of tHcy has emerged as an important risk factor in the assessment of cardiovascular disease.[1,2,3] Excess Hcy in the blood stream may cause injuries to arterial vessels due to its irritant nature, and result in inflammation and plaque formation, which may eventually cause blockage of 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 recently 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^+ ($\Delta A_{340\,nm}$).



NH_3 + NADH + 2-Oxoglutarate $\xrightarrow{GLDH}$ Glutamate + NAD^+ + H_2O

Reagents - working solutions

R1 **NADH reagent**
S-adenosylmethionine 0.1 mmol/L; TCEP[a)] > 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

SR **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

a) Tris(2-carboxyethyl)phosphine

R1 is in position A, R2 is in position B and SR is in position C.

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
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	4 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	4 weeks

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.

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

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350 322, system-ID 07 5635 0 for automatic postdilution and standard serial dilutions. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus/800 analyzers.

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.

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1/R2-S-SR
Reaction direction	Decrease
Wavelength A/B	340/659 nm
Calc. first/last	50/62
Unit	µmol/L

Pipetting parameters

		Diluent (H_2O)
R1	175 µL	–
R2	27 µL	–
Sample	14 µL	–
SR	18 µL	–
Total volume	234 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1/R2-S-SR
Reaction direction	Decrease
Wavelength A/B	340/659 nm
Calc. first/last	73/95
Unit	µmol/L

Pipetting parameters

		Diluent (H_2O)
R1	175 µL	–
R2	27 µL	–
Sample	14 µL	–
SR	18 µL	–
Total volume	234 µL	

Calibration

Calibrators	HCYS Calibrator Kit Calibration dilution ratio: 1:1, 1:2, 1:4, 1:8, 1:18 performed automatically by the instrument
Calibration mode	Logit/log 5
Calibration replicate	Duplicate recommended
Calibration frequency	Full calibration ▪ every 7 days ▪ after reagent lot change ▪ 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

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Limitations - interference

Criterion: Recovery within ± 1.5 µmol/L of initial values of samples ≤ 15 µmol/L and within ± 10 % for samples > 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 metabolic 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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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

The lower and the upper limit of the measuring range depends on the actual calibrator value.

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-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 µmol/L*	*SD µmol/L*	*CV %*
Homocystein Control 1	12.2	0.1	1.0
Homocystein Control 2	38.9	0.5	1.3
Human serum 1	8.47	0.09	1.1
Human serum 2	13.5	0.1	0.9
Human serum 3	31.2	0.3	0.9
Human serum 4	45.5	0.6	1.4

Intermediate precision	*Mean µmol/L*	*SD µmol/L*	*CV %*
Homocystein Control 1	12.2	0.2	1.4
Homocystein Control 2	38.9	0.6	1.5
Human serum 1	8.47	0.11	1.3
Human serum 2	13.5	0.2	1.4
Human serum 3	31.2	0.5	1.4
Human serum 4	45.5	0.8	1.7

Method comparison

Hcy values for human serum samples obtained on a COBAS INTEGRA 800 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 = 1.00x + 0.144 µmol/L	y = 1.04x - 0.224 µmol/L
τ = 0.967	r = 0.998

The sample concentrations were between 3.39 and 46.8 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.

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

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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 after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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