Homocysteine FS*

Diagnostic reagent for quantitative in vitro determination of Homocysteine in serum or plasma on photometric systems

Order Information

Cat. No.	Kit size
1 3409 99 10 930	R1 4 x 12.5 mL + R2 1 x 8 mL + R3 1 x 6 mL
1 3400 99 10 041	3 x 1 mL TruCal Homocysteine

Summary [1]

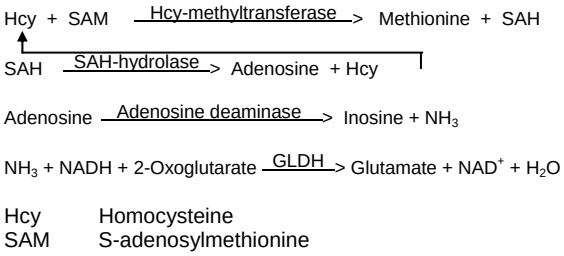
Homocysteine is a sulfur-containing amino acid which exists as intermediate in the methionine cycle. Increased plasma homocysteine is a sensitive marker of folate and cobalamin (vitamin B_{12}) deficiency and an independent risk factor for cardiovascular disease (CVD). Increased homocysteine concentrations are also related to birth defects, pregnancy complications, psychiatric disorders and cognitive impairment in the elderly.

Method

Enzymatic cycling method

Principle

Oxidized total homocysteine is reduced to free homocysteine (Hcy). The free Hcy reacts with a co-substrate, S-adenosylmethionine (SAM) catalyzed by a Hcy S-methyltransferase to form methionine and S-adenosyl homocysteine (SAH). SAH is hydrolysed into adenosine and Hcy by SAH-hydrolase. The formed Hcy is cycled into the Hcy conversion reaction by Hcy-S-methyltransferase. The cycling reaction leads to significant amplification of detection signals.
The formed adenosine is immediately hydrolysed into inosine and ammonia which is processed by glutamate dehydrogenase with concomitant conversion of NADH to NAD^+. The decrease of NADH is measured at 340 nm and is proportional to the homocysteine concentration in the sample.



Hcy	Homocysteine
SAM	S-adenosylmethionine
SAH	S-adenosylhomocysteine
GLDH	Glutamate dehydrogenase

Reagents

Components and Concentrations

S-adenosylmethionine (SAM)	0.1 mmol/L
NADH	0.2 mmol/L
TCEP	0.5 mmol/L
2-oxoglutarate	5.0 mmol/L
Glutamate dehydrogenase	10 kU/L
SAH hydrolase	3.0 kU/L
Adenosine deaminase	5.0 kU/L
Hcy methyltransferase	5.0 kU/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. The reagents contain sodium azide (< 1 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. In very rare cases, samples of patients with gammopathy might give falsified results [6].
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
4. Do not mix reagents of different lots.
5. The reagents should be clear. They should be discarded if they become turbid or the initial absorbance of R1 is less than 0.5 at 340 nm (optical path 0.6 cm).
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

3-reagent system

The reagents are ready to use.

2-reagent system

Add 2 mL R2 to 12.5 mL R1; mix well and avoid foam formation. The mixture is stable one week at 2 – 8 °C if protected from light.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, EDTA and heparin plasma
Separate the plasma immediately after blood collection from cellular contents. Do not use lipemic or hemolytic samples.

Stability in serum and plasma [2]

4 days	at	20 – 25 °C
4 weeks	at	4 – 8 °C
4 years	at	–20 °C

Freeze only once! Discard contaminated specimens!

Assay Procedure for Analyzers

Application sheets for automated systems are available on request.

3-reagent system

Basic parameter for Hitachi 911

Wavelength	700/340 nm (bichromatic)
Temperature	37°C
Measurement	2-Point End (Fixed Time Kinetics)
Sample/Calibrator	15 µL
Reagent 1	200 µL
Reagent 2	32 µL
Reagent 3	20 µL
Addition Reagent 2	Cycle 5 (80 s)
Addition Reagent 3	Cycle 15 (276 s)
Absorbance 1	Cycle 23 (460 s)
Absorbance 2	Cycle 31 (590 s)
Calibration	linear

2-reagent system

Basic parameter for Hitachi 911

Wavelength	700/340 nm (bichromatic)
Temperature	37°C
Measurement	2-Point End (Fixed Time Kinetics)
Sample/Calibrator	15 µL
Reagent 1+2 (premixed)	230 µL
Reagent 3	20 µL
Addition Reagent 3	Cycle 15 (276 s)
Absorbance 1	Cycle 23 (460 s)
Absorbance 2	Cycle 31 (590 s)
Calibration	linear

Note: For manual procedures the volumes of sample, calibrator and reagents have to be calculated accordingly.

Calculation/Calibration

The concentration of homocysteine in unknown samples is derived from linear calibration. The calibration curve is obtained using TruCal Homocysteine calibrators Level 1 and 2. For Cobas Mira use TruCal Homocysteine Level 0 (Cobas Mira blank solution) and Level 2. The assigned values of the calibrators have been made traceable to the reference material NIST SRM 1955. When using the 3-reagent system, calibration is stable for at least five days. Daily calibration is needed when using the 2-reagent system.

Controls

For internal quality control, DiaSys TruLab Homocysteine controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruLab Homocysteine Level 1	5 9770 99 10 046	3 x 1 mL
TruLab Homocysteine Level 2	5 9780 99 10 046	3 x 1 mL

Performance Characteristics

Measuring range

The test has been developed to determine concentrations of homocysteine up to 50 µmol/L. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences

No interference is observed by cystathionine up to 20 µmol/L, adenosine up to 100 µmol/L, L-cysteine up to 1 mmol/L, glutathione up to 500 µmol/L, hemoglobin up to 1200 mg/dL, ascorbic acid up to 10 mmol/L, bilirubin up to 20 mg/dL, lipemia up to 2500 mg/dL triglycerides, sodium fluoride up to 1 mmol/L, sodium phosphate up to 1 mmol/L and ammonium up to 500 µmol/L. For further information on interfering substances refer to Young DS [3].

Sensitivity/Limit of Detection

The lower limit of detection is 1 µmol/L.

Precision

According to protocol EP-5 of the NCCLS (National Committee of Clinical Laboratory Standards)

Within run precision	n	Mean [µmol/L]	SD [µmol/L]	CV [%]
Sample 1	40	7.2	0.16	2.2
Sample 2	80	13.2	0.64	3.0
Sample 3	80	29.1	0.81	1.8

Total precision	n	Mean [µmol/L]	SD [µmol/L]	CV [%]
Sample 1	40	7.2	0.29	4.1
Sample 2	80	13.2	0.72	5.9
Sample 3	80	29.1	0.92	4.0

Method Comparison

A comparison of DiaSys Homocysteine FS (y) with a commercially available test (x) using 72 samples gave following results:
y = 1.013 x – 0.162 µmol/L; r= 0.978.

Reference Range [4,5]

Women:	
< 30 years	6 – 14 µmol/L
30 – 59 years	5 – 13 µmol/L
> 60 years	7 – 14 µmol/L
Men:	
< 30 years	6 – 14 µmol/L
30 – 59 years	6 – 16 µmol/L
> 60 years	6 – 17 µmol/L
> 85 years	15 – 30 µmol/L

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Refsum H, Smith AD, Ueland PM, Nexo E et al. Facts and recommendations about Total Homocysteine Determinations: an Expert Opinion. Clin Chem 2004; 50: 3–32.
2. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 34-5.
3. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
4. Schreiner H, Göbel-Schreiner B, Durst C, Casper R, Walch S. Homocysteine: reference values. Clin Lab 1997; 43: 1121-4.
5. Herrmann W, Quast S, Ullrich M, Schultze H, Geisel J. The importance of hyperhomocysteinemia in high age people. Clin Lab 1997; 43: 1005-9.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem Lab Med 2007; 45(9): 1240–1243.

Manufacturer

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