DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
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Liquid reagents – ready to use

Homocysteine

Enzymatic cycling

2 Reagents

Diagnostic reagent for the quantitative in vitro determination of total L-Homocysteine in human serum or plasma on photometric systems

REF	Kit Size	Content
908510	**4 x 20 mL**	3 x 20 mL R1 + 1 x 18 mL R2
908520	**4 x 10 mL**	3 x 10 mL R1 + 1 x 9 mL R2
992911	**1 x 39 mL**	1 x 30 mL R1 + 1 x 9 mL R2
9A0849	**4 x 20 mL**	3 x 20 mL R1 + 1 x 18 mL R2
DT1034	**4 x 20 mL**	4 x 20 mL R1 + 1 x 20 mL R2
9T1049	**4 x 20 mL**	4 x 20 mL R1 + 1 x 20 mL R2
9E1849	**2 x 39 mL**	2 x 30 mL R1 + 2 x 9 mL R2

Additionally offered:

908550	5 x 1 mL	Homocysteine Calibrator Set (5 levels)
905620	4 x 1 mL	Homocysteine Control Set (4 levels)

TESTPARAMETER

Method:	UV, 2 point kinetic (fixed time) reaction, enzymatic cycling
Wavelength:	340 nm
Temperature:	37 °C
Sample:	Serum, heparin plasma, EDTA plasma
Linearity:	up to 50 µmol/L
Sensitivity:	The lower limit of detection is 0.4 µmol/L

SUMMARY

Elevated level of total Homocysteine (tHcy) has emerged as an important risk factor in the assessment of cardiovascular disease [1-3]. Excess homocysteine (Hcy) in the bloodstream 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 levels of tHcy are also linked with Alzheimer's disease [4] and osteoporosis [5].

TEST PRINCIPLE

Hcy + SAM $\xrightarrow{\text{Hcy-methyltransferase}}$ Methionine + SAH

SAH $\xrightarrow{\text{SAH-hydrolase}}$ Adenosine + **Hcy**

Adenosine $\xrightarrow{\text{Adenosine deaminase}}$ Inosine + NH_3

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

In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM), catalyzed by a Hcy S-methyltransferase to form methionine (the Hcy converson product of Hcy) and S-adenosylhomocysteine (SAH, the co-substrate conversion product). SAH is assessed by coupled enzyme reactons including SAH hydrolase, adenosine deaminase and glutamate dehydrogenase, wherein SAH is hydrolyzed into adenosine and Hcy by SAH hydrolase. The formed Hcy that is originated from the co-substrate SAM is cycled into the Hcy conversion reaction by Hcy S-methyltransferase. This forms a co-substrate conversion product based enzyme cycling reaction system with significant amplification of detection signals. The formed adenosine is immediately 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^+.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
S-Adenosylmethionine (SAM)	0.1 mM
NADH	> 0.2 mM
TCEP	> 0.5 mM
2-Oxoglutarate	5.0 mM
Glutamate dehydrogenase	10 KU/L
SAH hydrolase	3.0 KU/L
Adenosine deaminase	5.0 KU/L
Hcy methyltransferase	5.0 KU/L

REAGENT PREPARATION

The reagents are ready to use.

REAGENT STABILITY AND STORAGE

Conditions: Protect from light.
Close immediately after use. Avoid contamination.
Do not freeze!
Storage: at 2 – 8 °C
Stability: Up to the expiration date

The reagent should be clear. It should be discarded if it becomes turbid or the initial absorbance is less than 0.5 at 340 nm (light path 0.6).

SAMPLE COLLECTION AND HANDLING

Fresh serum, heparin plasma, or EDTA plasma can be used in the Homocysteine assay.
Centrifuge blood sample immediately after collection! If immediate centrifugation is not possible, collected blood specimens should be kept on ice and centrifuged within an hour.
Discard hemolysed, turbid or severely lipemic specimens.
Addition of 3-deazaadenosine to inhibit Hcy production in red cells has been suggested. However, the Dialab Hcy assay cannot use samples containing 3-daazaadenosine since it inhibits one of the key enzymes used in the assay.

SAMPLE STABILITY AND STORAGE

Serum, Heparin plasma or EDTA Plasma:

At room temperature:	4 days
at 0 – 8 °C	several weeks
at - 20 °C	> 1 year

MATERIALS REQUIRED BUT NOT PROVIDED

Analyzer capable of measuring absorbance at 340 nm and temperature control
General laboratory equipment

ASSAY PROCEDURE*

Main wavelength: 340 nm
Second wavelength: 700 nm
Sample: 13 µl
Reagent 1: 240 µl
Incubation Time: 1 – 5 minutes
Reagent 2: 65 µl
1st Reading: 3 – 5 minutes after adding R2
2nd Reading: 3 – 5 minutes after 1st reading
* depending on instrument

REFERENCE RANGE

In most clinical laboratories, a concentration between 12 and 15 µmol/L is used as the cut-off value for normal level of Hcy for adults.
However, each laboratory is recommended to establishes a range of normal values for the population in their region.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

The test has been developed to determine homocysteine concentrations within a measuring range from 3 - 50 µmol/L. Samples with values higher than 50 µmol/L should be diluted 1:2 with distilled water and rerun. Multiply results by 2.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 0.4 µmol/L.

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PRECISION

Within run precision, n=20	Mean [µmol/L]	SD [µmol/L]	CV [%]
Sample 1	7.08	0.33	4.61
Sample 2	12.4	0.24	1.91
Sample 3	16.2	0.51	3.12
Sample 4	26.9	0.66	2.47

Inter run precision, n=30	Mean [µmol/L]	SD [µmol/L]	CV [%]
Sample 1	7.32	0.44	5.98
Sample 2	11.3	0.57	5.08
Sample 3	14.4	0.81	5.61
Sample 4	27.7	0.72	2.61

SPECIFICITY/INTERFERENCES

The following substances normally present in the serum produced less than 10% deviation when tested at levels equal to the concentrations listed below.

Ascorbic Acid	10 mM
Bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
Triglyceride	1000 mg/dL
Cystathionine	100 µM

Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azuridine triacetate may have higher levels of homocysteine due to metabolic interference with homocysteine metabolism.
S-adenosylhomocysteine (SAH) will cause a significant positive interference. However, SAH is either not detectable or at very low concentrations in normal plasma and should not cause concern.
Automated chemistry analyzers use on-board routine wash steps to prevent reagent carry-over by reagent probes. However, the efficiency of the routine reagent probe wash varies and additional wash steps may be needed.

METHOD COMPARISON

Correlation studies were performed by testing 40 serum samples in comparison with an existing commercial Hcy assay method. Linear regression gives the following equation:
$y = 0.94\ x + 1.05$; $R^2 = 0.99$

CALIBRATION

The assay requires the use of an Homocysteine Standard or Calibrator.
We recommend the Dialab **Homocysteine Calibrator Set** (5 levels).

QUALITY CONTROL

Homocysteine controls should be used to validate the performance of Hcy reagents.
We recommend the Dialab **Homocysteine Control Set** (4 leveles).
Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION

Special adaptations for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

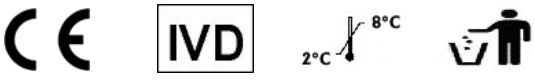
1. For in vitro diagnostic use by suitably qualified laboratory personnel only
2. Do not ingest! Avoid contact with skin, eyes and mucous membranes.
3. Contains sodium azide which may react with lead or copper plumbing to form explosive compounds. Flush drains with copious amounts of water when disposing of this reagent.
4. Do not mix reagents of different lots.
5. Take the necessary precautions for the use of laboratory reagents.
6. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

1. Eikelboom JW, et al. *Ann Intern Med* 131:363-75 (1999)
2. Scott J, Weir D. Q *J Med* 89: 561-3 (1996)
3. Nygard L, *N Engl J Med.* 337(4):230-6 (1997)
4. Seshadri S. *et al. N. Engl. J. Med.* 346:477-483 (2002)
5. McLean R. et al.*N. Engl. J. Med.* 350:2042-2049 (2004)
6. Refsum H. *Clinical Laboratory News* May 2002, pp 2-14
7. Guttormsen AB et al. *J Nutr.* 124(10): 193-41 (1994)
8. Vilaseca et al. *Clin. Chem.* 43:690-692 (1997)
9. Faure-Delanef et al. *Am. J. Hum. Genet.* 60:999-1001 (1997)



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