HOMOCYSTEIN (HCY)

Diagnostic reagent for determination of Homocystein concentration.

Liquid. Dual Reagents. Store at 2°C - 8°C. For in Vitro Diagnostic Use. Do not freeze.

Ref No	Pack	Ref No	Pack	Ref No	Pack	Ref No	Pack
ZA51	3*25 ML						

INTENDED USE

The Archem Homocysteine Enzymatic Assay kit is intended for the *in vitro* quantitative determination of total L-homocysteine in serum or plasma.
The assay can assist in diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria. The assay is not intended for correlating B12 or folate with homocysteine levels.
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.

CLINICAL SIGNIFICANCE

Homocysteine (Hcy) is a thiol-containing amino acid produced by the intracellu-lar demethylation of methionine. Total homocysteine (tHcy) represents the sum of all forms of Hcy including forms of oxidized, protein bound and free.
Elevated level of tHcy has emerged as an important risk factor in the assessment of cardiovascular disease. Excess 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. Ele-vated tHcy levels are caused by four major factors, including: a) genetic deficien-cies in enzymes involved in Hcy metabolisms such as cystathionine beta-synthase (CBS), methionine synthase (MS), and methylenetetrahydrofolate reductase (MTHFR); b) nutritional deficiency in B vitamins such as B_6, B_{12} and folate; c) renal failure for effective amino acid clearance, and d) drug interactions such as nitric oxide, methotrexate and phenytoin that interfere with Hcy metabolisms. Elevated levels of tHcy are also linked with Alzheimer's disease and osteoporo-sis. Guidelines for tHcy determination in clinical laboratories have recently been established.

TEST PRINCIPLE

Enzymatic tHcy assay is based on a novel assay principle that assesses the co-substrate conversion product (a molecule that is not a substrate of the Hcy conversion enzyme, and does not contain any element from sample Hcy) instead of assessing co-substrate or Hcy conversion products of Hcy as One described in the literature. In this assay, oxidized. Hcy is 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 conversion product of Hcy) and S-adenosylhomocysteine (SAH, the co-substrate conversion product). SAH is assessed by coupled enzyme reactions including SAH hydrolase, adenosine (Ado) deaminase and glutamate dehydrogenase wherein SAH is hydrolyzed into adenosine (Ado) and Hcy by SAH hydrolase. The formed Hcy that is originated from the co-substrate SAM is cycled into the Hc y 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 Ado is immediately hydrolyzed into Inosine and ammonia which reacts with glutamate dehydrogenase with concomitant conversions of NADH to NAD+. The concentration of Hcy in the sample is indirectly proportional to the amount of NADH converted to NAD+ (A340nm).

TEST PARAMETERS

Method	: Colorimetric, Kinetic, Increasing Reaction
Wavelength	: Main:34 0nm Sub:700
Temperature	: 37°C
Sample	: Serum, heparin Plasma
Linearity	: 50 µmol/L

REAGENT COMPOSITION

R1&R2:

Glutamate dehydrogenase	≤4 0 KU/L
SAH hydrolase	≤4.0 KU/L
Adenosine deaminase	v5.0 KU/L
TCEP	> 0.4 mmol/L
Hcy methyltransferase	>0.15 mmol/L
NADH	> 0.15 mmol/L
S-adenosylmethionine (SAM)	≤0.1 mmol/L

REAGENT PREPARATION

Sample Start:
Mix 3 parts of Reagent 1 (Buffer) with 1 part of Reagent 2 (Starter). Avoid foaming.
Stability of working reagent: 2 days at 2-8°C, away from light sources.

Working reagents are stable at 2-8ºC in case of closed vials and avoiding contamination after preparation.

For manual working procedures; if working reagent will be used; shake the Reagent 2 vial gently before pouring its contents into the Reagent 1 bottle. It is advisable to wash the Reagent 2 vial with a small volume of the prepared mixture in order to completely rinse the vial and avoid any losses.

REAGENT STABILITY AND STORAGE

Once opened vials are stable minimum 30 days at 2-8 C at optimum conditions. On board stability is strongly related to auto analyzers cooling specification and carry-over values.

SAMPLE

Fresh serum or heparin plasma are the recommended samples for the Hcy assay. EDTA plasma samples can also be used. 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 Hcy assay. After separation of plasma from cells, Hcy is stable for at least 4 days at room temperature and stable for several weeks at 0-8°C, and stable for several months or years at -20°C.

TEST PROCEDURE

Sample Start
There have many ready application procedures dedicated to different kind of photometers and ready manual working process can be supplied on request.
There have many ready application procedures dedicated to different kind of biochemistry auto analyzers can be supplied on request.

Substrate Start
There have many ready application procedures dedicated to different kind of biochemistry auto analyzers can be supplied on request.

CALCULATION

For Sample Start:
ΔA/min x Factor = µmol/L Homocystein in sample

REFERENCE INTERVAL (NORMAL VALUES)

Adult: 0 - 15 µmol/L

*It is recommended that each laboratory establish its own reference range.

QUALITY CONTROL AND CALIBRATION

Some control sera with Homocystein values determined by this method can be used. We recommend:

ZA68	HCY Calibrator	5*1 ML(SET)
ZA69	HCY Control Level 1-2	5*1 ML+5*1 ML(SET)

*Calibration Stability: It is strongly depend of application to auto analyzers and auto analyzers specification. Calibration stability is **at least** 5 days.

*Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

Quality control is recommended every morning. Calibration is not recommended if QC control values are acceptable. Reagent should be calibrated after lot changes.

PERFORMANCE CHARACTERISTICS

Low linearity: 1,5 µmol/L

High Linearity: The method is linear up to 200 U/l.

If a ΔA/min of 0.500 is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Linearity may considerably vary depending on the instrument used.

Precision Studies (Based on CLSI EP5 Doc.):

Intra assay CV% < 6.0%, Inter assay CV% < 4.5%

Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

Methods comparison: A comparison between Archem and a commercially available product gave the following results:

HCY Archem = x
HCY competitor = y
$r^2 = 0.99$

Interferences: No interference was observed by the presence of:

Hemoglobin	≤ 500 mg/dL
Bilirubin	≤ 40 mg/dL
Trigliserid	≤ 2500 mg/dL

These performance characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure is used.

NOTES

1. For in vitro diagnostic use only. Do not pipette by mouth. Avoid contact with skin and mucous membranes.
2. All the calibrators, controls and some reagents must be considered as human & animal sample, so potentially infectious; all the protection actions must be applied to avoid any potential biological risk.
3. Material safety data sheet will be supplied on request.
4. Exercise the normal precautions required for handling laboratory reagents.
5. After measurements are taken, reagent bottles should cap and kept at 2-8°C. Caps of the reagents bottles cannot be used between two different kind of reagent and between R1&R2.
6. Reagents with different lot numbers should not be interchanged or mixed.
7. The linearity limit depends on the sample to reagent ratio.
8. Assay is specific for HCY and has no detectable reaction with other nucleosides

PRECAUTIONS AND WASTE DISPOSAL

This product is made to be used in professional laboratories and by professional operators. Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.
R36/38 : Irritating to eyes and skin.
S20/21 : When using, do not eat, drink or smoke.
S26 : In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 : After contact with skin wash immediately with plenty of water.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S45 : In case of accident or if you feel unwell, seek medical advice immediately.
S56 : Dispose of this material and its container at hazardous or special waste collection point.
S57 : Use appropriate container to avoid environmental contamination.
S61 : Avoid release in environment. Refer to special instructions/safety data sheets.
Please consult local regulations for a correct waste disposal.

ABBREVIATIONS

HCY : Homocystein
CLSI : Clinical and Laboratory Standards Institute
CV% : Coefficient of Variation Percentage
EP : Evaluation Protocols
GLP : Good Laboratory Practice
IU : International Unit
mA : miliabsorbance
mL : mililiter
NCCLS : National Committee for Clinical Laboratory Standards
QC : Quality Control

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SYMBOLS

IVD	Only for invitro diagnostic use
LOT	Lot of manufacturing
R1	Reagent 1
R2	Reagent 2
CONC	Concentration
INGRED	Reagent Ingredients
REF	Reference Number (Catalog No)
SN	Serial Number
	Expiration date
	Storage temperature interval
	Read the directions
	Biological risk

CE

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