# HOMOCYSTEINE Enzymatic

**Enzymatic test for the quantitative assay of Homocysteine (HCY) in serum and plasma**

CE IVD

## ORDER INFORMATION

| REF | Kit size |
|---|---|
| GA4405 00 | 1x12.5 + 1x3.5 ml |
| GA4415 00 | 1x25 + 1x7 ml |
| GA4410 00 | 2x25 + 2x7 ml |
| KL4410 00 | 2x25 + 2x7 ml |
| BK4410 00 | 1x(50+14 ml) |

## INDICATION

Homocysteine (HCY) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Total homocysteine T(HCY) represents the sum of all forms of HCY (including forms of oxidized, protein bound and free).

Elevated level of Homocysteine has emerged as an important risk factor in the assessment of cardiovascular disease[1-3]. 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.

Elevated Homocysteine levels are caused by four major factors, including:

1. genetic deficiencies in enzymes involved in HCY metabolisms such as cystathionine beta-synthase (CBS), methionine synthase (MS), and methylenetetrahydrofolate reductase (MTHFR);
2. nutritional deficiency in B vitamins (B6, B12 and folate);
3. renal failure for effective amino acid clearance;
4. drug interactions such as nitric oxide, methotrexate and phenytoin that interfere with HCY metabolisms.

Elevated levels of T(HCY) are also linked with Alzheimer's disease[4] and osteoporosis[5]. Guidelines for T(HCY) determination in clinical laboratories have recently been established[6].

## METHOD PRINCIPLE

The AMS enzymatic test for the quantitative Homocysteine determination (HCY) is based on a series of enzymatic reactions causing a decrease in absorbance value due to NADH oxidation to $NAD^+$. HCY concentration in the sample is directly proportional to the quantity of NADH converted to $NAD^+$ ($\Delta A_{340nm}$). The enzymatic reactions are the following:

T(HCY) → (HCY)free

(HCY)free + SAM* —(s-Methyltransferase)→ Methionine + SAH**

SAH + NADH —(SAM Hydrolase + Adenosine deaminase / Glutamatedehydrogenase)→ Inosine + $NAD^+$

* SAM = S-Adenosyl-methionine
** SAH = S-Adenosyl-homocysteine

## COMPOSITION

**REAGENT A**:

| | |
|---|---|
| S-adenosylmethionine | 0.1 mmol/l |
| NADH | 0.2 mmol/l |
| TCEP | 0.5 mmol/l |
| 2-oxoglutarate | 5.0 mmol/l |

**REAGENT B**:

| | |
|---|---|
| Glutamate dehydrogenase | 10 KU/l |
| SAH hydrolase | 3.0 KU/l |
| Adenosyne deaminase | 5.0 KU/l |
| HCY methyltransferase | 5.0 KU/l |

**CALIBRATORS**: 2 levels 2x1 ml

### Preparation

The reagents are liquids ready to use.

**Note:**
The reagent used should be clear. It should be discarded and can not be used if it becomes turbid or the initial absorbance is less than 0.5 at 340 nm.

### Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

## ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

## SAMPLES

Fresh serum or plasma is recommended sample for the HCY assay.

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 whithin an hour. Hemolysed or turbid specimens or severly lipemic specimens are not recommended for HCY assay.

Stability:
After separation of serum/plasma from cells, HCY is stable in the sample is stored for 4 days at room temperature, 4 weeks at 2-8 °C, and for 12 months at -20 °C.

### Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

## INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known Homocysteine concentration. Check that the values obtained are within the reference range provided.

## ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes:

| | Blank | CAL 1 | CAL 2 | Sample |
|---|---|---|---|---|
| Reagent A | 950 µl | 950 µl | 950 µl | 950 µl |
| Saline | 50 µl | - | - | - |
| Calibrator 1 | - | 50 µl | - | - |
| Calibrator 2 | - | - | 50 µl | - |
| Sample | - | - | - | 50 µl |
| Mix and incubate for 5 minutes at 37 °C. Then add: | | | | |
| Reagent B | 250 µl | 250 µl | 250 µl | 250 µl |
| Mix and incubate at 37 °C for 2.5 minutes. Read absorbance ($A_1$) at 340 nm and then read again after 2.5 minutes ($A_2$). | | | | |

**Note:**
- Reaction volumes can be proportionally changed.
- Samples with values greater than 50 µmol/l should be diluted 1:2 and tested again. Multiply results by 2.

## CALCULATION OF RESULTS
Plot the ΔA ($A_2$ - $A_1$) calculated for blank and each calibrator against its concentration (concentrations are reported on the calibrator vial label).
Results are found by comparing the sample ΔA against the plotted curve.
A curve fitting system software it is suggested to achieve more precise results.

## AUTOMATIC ANALYZERS
The present kit can be used with any type of Clinical Chemistry automatic analyzer. Applications parameters for the most commons commercially available instruments are available upon request (Hitachi, Olympus, Beckman, Konelab, etc.).

## CALIBRATION
Use the calibrator 1 and 2 for establish the calibration curve. Use saline or distilled water as calibration point 0 (0 µmol/l) where applicable or requested.
Two different values referring to two different standardizations are given for each calibrator:
- NIST: values standardized and traceable to NIST SRM 1955 "Homocysteine Standard Reference Material";
- CLIA: values standardized against chemiluminescent assays (Immulite, Bayer Advia Centaur®, Abbott AxSYM, etc.);

These two values and consequently results differ about 20%, having CLIA methods values lower than NIST methods. Each laboratory is responsible for the adopted standardization.
Assigned values are printed on the vials' label and are lot dependent.
Using only one calibrator (linear calibration) is an approximation of the actual calibration curve (non-linear calibration). Even if it is suggested to use both calibrators for establishing the calibration curve in order to achieve the best accuracy, using a linear calibration curve is possible, but it leads to a sensible reduction of the assay accuracy.
Calibration is stable up to 5 days. Anyway, it is suggested to recalibrate in case of the assay Quality Control gives non acceptable results.

## REFERENCE VALUES
NIST standardized study shows 15 µmol/l as the cut-off value for normal level of HCY for adults[8,9].
Suggested reference values accordingly to the different standardizations are given in the following table:

| Standardiztion | NIST | CLIA |
|---|---|---|
| Adult normal cut-off | ≤ 15 µmol/l | ≤ 12 µmol/l |

Each laboratory is recommended to establish a range of normal values for the population in their region.

## ANALYTICAL PERFORMANCES
### Sensitivity
Test sensitivity, in terms of limit of detection, is 0.4 µmol/l.

### Linearity
The assay is linear up to 50 µmol/l.

### Precision
Precision has been evaluated by testing replicates of four samples at different Homocysteine concentration.
The obtained results are reported in the following tables.

**Within-run precision**

| Sample | n | Mean (µmol/l) | SD | %CV |
|---|---|---|---|---|
| Sample 1 | 40 | 7.0 | 0.32 | 4.57 |
| Sample 2 | 40 | 12.0 | 0.22 | 1.83 |
| Sample 3 | 40 | 15.6 | 0.47 | 3.01 |
| Sample 4 | 40 | 29.0 | 0.70 | 2.41 |

**Between-run-precision**

| Sample | n | Mean (µmol/l) | SD | %CV |
|---|---|---|---|---|
| Sample 1 | 40 | 7.0 | 0.42 | 6.00 |
| Sample 2 | 40 | 12.0 | 0.59 | 4.92 |
| Sample 3 | 40 | 15.6 | 0.80 | 5.12 |
| Sample 4 | 40 | 29.0 | 0.75 | 2.59 |

### Correlation
A correlation study comparing the present method and a commercial one gave the following results:

y = 0.94x + 1.05 µmol/l r = 0.99

### Interferences
- Interferences were tested with a serum sample spiked with various concentrations of substances normally present in the serum. In the following table, concentrations of different substances with interferences less than 10% are reported:

| | |
|---|---|
| Bilirubin | 40 mg/dl |
| Bilirubin conjugated | 40 mg/dl |
| Triglycerides | 1000 mg/dl |
| Ascorbic acid | 10 mmol/l |
| Hemoglobin | 500 mg/dl |
| Cystathionine | 0.1 mmol/l |

- Patients taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azuridine triacetate may have high levels of HCY, due to the interference of this drugs with the Homocysteine metabolism.
- Addition of 3-deazaadenosine to inhibit HCY production in red cells has been suggested. However, the HCY assay can not use samples containing 3-deazaadenosine since it inhibits one of the key enzyme used in the assay.

## PRECAUTIONS IN USE
The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

### Waste Management
Please refer to local legal requirements.

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