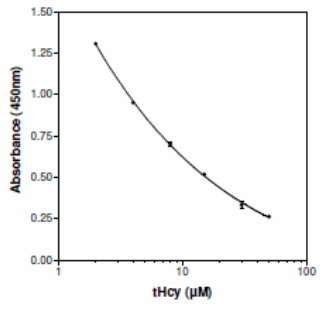
Homocysteine ELISA Kit, 96 tests Cat# 0370-HCY

The human Homocysteine ELISA kit is intended for the quantitative determination of total homocysteine in plasma or serum. Protein bound homocysteine is reduced to free homocysteine and enzymatically converted to S-adenosyl-L-homocysteine (SAH) prior to the immunoassay. *For research use only.*



Typical std. curve

ELISA Kit Features

- S-adenosyl-L-homocysteine (SAH) Pre-coated, stabilized, ready-to-use 96-well strip plate, suitable for multiple runs over 6-9 months.
- Human SAH stds (2, 4, 8, 15, 30, 50 umol/l).
- Low, medium and high controls. (6-25 umol)
- 25 ul samples
- 2 hr assay, 3 incubation steps at 18-25 °C.
- Contains all necessary reagents. Stability ~12 months
- For in vitro research use only.

Sample pretreatment: Allow all reagents to reach room temperature.

Sample pre-treatment solution must be made up no more than 1 hour prior to the start of the assay.

Step1 **Mix 4.5 ml of** Assay Buffer, 0.25 ml of Adenosine/DTT and 0.25 ml SAH-hydrolase in a tube for the pretreatment solution
Step 2 **Pipette 25 ul of** standards, samples and controls into 500 ul of pretreatment solution. Incubate at 37 C for 30 mins.
Step 3 **Add 500 ul of** Enzyme Inhibitor, Mix and incubate for 15 C at RT.
Step 3 **Add 500 ul of** Adenosine deaminase to the solution, Mix and incubate for 15 C at RT.

Assay Procedure: Allow all reagents to reach room temperature.

Step 1. Pipet **25 ul** each of pre-diluted controls, standards, samples into precoated wells. Cover and incubate for **30 mins at 18-25 °C.**
Step 2 Add 200 ul of **anti SAH antibody** to each well. Incubate for **30 mins at 18-25 °C.**
Step 3. Aspirate and wash 3X. Add **100 ul** of **enzyme conjugate.** to all wells. Incubate at **30 mins at 18-25 °C.**
Step 3. Aspirate and wash 3X. Add **100 ul** of **TMB substrate** to all wells. Incubate for 10 **mins at 18-25 °C.**
Step 4. Add **100 ul** of **stop** solution into each well and mix gently (blue color turns yellow). Measure absorbance at 450 nm. Determine Antibody conc. in each sample using the standards

Interpretation of results

The mean values for the measured absorptions are calculated after subtraction of the blank values from the controls and standards.

Precision: **Intra-assay (%):** 5.2-7.3 % **Total (%):** 7.1-9.3

Limit of quantification: 1.0 µmol/L with a CV < 20%.

Linearity: %recovery: 90-110

Interfering substances: < 10 % for Bilirubin, hemoglobin, lipids, protein and red blood cells.

Cross reactivity: Adenosyl-L-methionine (SAM) (16.3%), Adenosine, Cystathionine, LCysteine, Gluthathione and Thiolactone (<10%)

General Information

Homocysteine is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Homocysteine is metabolised to either cysteine or to methionine. In the vitamin B6 dependent trans-sulphuration pathway homocysteine is irreversibly catabolised to cysteine. A major part of homocysteine is remethylated to methionine, mainly by the folate and cobalamin dependent enzyme methionine synthase. Homocysteine accumulates in the cell and is exported to the circulation when these reactions are impaired. Homocysteine circulates in plasma mostly in its oxidised form bound to proteins and is measured as total homocysteine, (tHcy), the sum of free and protein bound. Severely elevated concentrations of tHcy are found in subjects with homocystinuria, a rare genetic disorder of the enzymes involved in the metabolism of homocysteine. Patients with homocystinuria exhibit mental retardation, early arteriosclerosis and arterial and venous thromboembolism.
Epidemiological research has shown that different patient groups might have elevated homocysteine levels in blood. More than 40 epidemiological studies have shown that elevated tHcy is an independent risk factor of cardiovascular disease (CVD). A meta analysis of 27 of these studies, including more than 4000 patients, estimated that a 5 µmol/L increase in tHcy was associated with an odds ratio of 1.7 for coronary artery disease, 1.5 for cerebrovascular disease or the same increase in risk as for 0.5 mmol/L increase in cholesterol9. Peripheral arterial disease also showed a strong association. Patients with chronic renal disease experiences an excess morbidity and mortality due to arteriosclerotic CVD. Elevated concentration of tHcy is a frequent observed finding in the blood of these patients. Although they may lack some of the vitamins involved in the metabolism of homocysteine, the increased levels of tHcy are mainly due to impaired removal of homocysteine from the blood by the kidney. Drugs that interfere with the homocysteine metabolism, e.g. nitric oxide, methotrexate, isoniacid, penicillamine and various antiepileptic drugs, may give elevated levels of total homocysteine.

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