

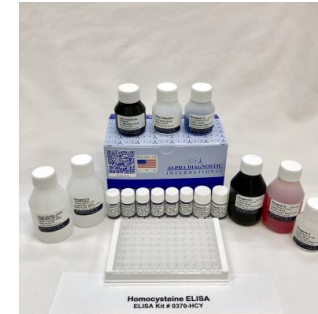
ELISA kits available from ADI (see details at the web site)

- 3300-200-B2A** Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests
- 3300-210-PSS** Human Anti-Phosphatidyl serine IgG/IgM ELISA kit, 96 tests
- 3300-215-PIS** Human Anti-Phosphatidyl Inositol IgG/IgM ELISA kit, 96 tests
- 3300-220-PAS** Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests
- 3300-230-APG** Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests
- 3300-235-APA** Human Anti-Prothrombin IgA ELISA kit, 96 tests
- 3300-240-AVA** Human Anti-Annexin V IgG ELISA kit, 96 tests
- 3300-250-ANG** Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit
- 3300-255-PRG** Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
- 3300-260-LFG** Human Anti-Lactoferrin IgG ELISA kit, 96 tests, Quantitative
- 3300-265-MPG** Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
- 3300-290-ELG** Human Anti-Elastase IgG ELISA kit, 96 tests, Quantitative
- 3300-300-GLG** Human Anti-Gliadin IgG ELISA kit, 96 tests, Quantitative
- 3300-305-GLM** Human Anti-Gliadin IgM ELISA kit, 96 tests, Quantitative
- 3300-310-GLA** Human Anti-Gliadin IgA ELISA kit, 96 tests, Quantitative
- 3300-350-TPG** Human Anti-thyroid peroxidase (TPO) IgG ELISA kit
- 3300-360-TGG** Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests
- 3300-410-HTA** Human Anti-Toxoplasma IgA (Toxo-IgA) ELISA kit, 96 tests
- 3300-415-HTG** Human Anti-Toxoplasma IgG (Toxo-IgG) ELISA kit, 96 tests
- 3300-420-HTM** Human Anti-Toxoplasma IgM (Toxo-IgM) ELISA kit
- 3300-500-HCA** Human Anti-Chlamydia Trachomatis IgA ELISA kit, 96 tests
- 3300-510-HCG** Human Anti-Chlamydia Trachomatis IgG ELISA kit, 96 tests
- 3300-520-HCM** Human Anti-Chlamydia Trachomatis IgM ELISA kit, 96 tests

Instruction Manual No. M-0370-HCY

**Homocysteine ELISA kit
Cat. No. 0370-HCY, 96 Tests**

For Quantitative Determination of Homocysteine



For In Vitro Research Use Only



**ALPHA DIAGNOSTIC
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Homocysteine ELISA # 0370-HCY

For Quantitative Determination of homocysteine

Kit Contents: (reagents for 96 tests)

Components	
S-adenosyl-L-homocysteine coated microwell strips (12 X 8 wells), Ready-to-use, 0370P	1 Plate
S-adenosyl-L-homocysteine Standard A (2 µmol/L) in Assay buffer with preservative 1.5 ml, #0371A	1 bottle
S-adenosyl-L-homocysteine Standard B (4 µmol/L) in Assay buffer with preservative 1.5 ml, Ready-to-use, #0371B	1 bottle
S-adenosyl-L-homocysteine Standard C (8 µmol/L) in Assay buffer with preservative 1.5 ml, Ready-to-use, #0371C	1 bottle
S-adenosyl-L-homocysteine Standard D (15 µmol/L) in Assay buffer with preservative 1.5 ml, Ready-to-use, #0371D	1 bottle
S-adenosyl-L-homocysteine Standard E (30 µmol/L) in Assay buffer with preservative 1.5 ml, Ready-to-use, #0371E	1 bottle
S-adenosyl-L-homocysteine Standard F (50 µmol/L) in Assay buffer with preservative 1.5 ml, Ready-to-use, #0371F	1 bottle
Reagent A Assay buffer (Phosphate buffer, 0.09% NaN ₃), 54 ml, Ready-to-use, #0370-AB	1 bottle
Reagent B Adenosine/DTT, 3.5 ml, Ready-to-use, #0372	1 bottle
Reagent C SAH-Hydrolase, 3.5 ml, Ready-to-use, #0373	1 bottle
Reagent D Enzyme inhibitor, 55 mL, Ready to use. #0374	1 bottle (Dark bottle)
Reagent E Adenosine deaminase, 55 mL, Ready to use. #0375	1 bottle (Red coloured)
Reagent F Monoclonal mouse-anti-S-adenosyl-L-homocysteine-antibody, 25 mL, Ready to use. #0376	1 bottle
Reagent G Rabbit Anti-mouse-antibody enzyme conjugate, 15 mL, Ready to use. #0377	1 bottle (Blue coloured)
Wash buffer (10X) , 60 ml, # WB-0370 dilute 1:10 with distilled water before use.	1 bottle
HRP Substrate Solution, 15 ml, #TMB-0370	1 bottle
Stop solution, 20 ml, #ST-0370	1 bottle
Complete Instruction Manual; M-0370-HCY	1

Intended Use:

Homocysteine ELISA kit is intended for the Quantitative Determination of total L-homocysteine in plasma or serum. **For In Vitro Research Use Only (RUO).**

Introduction

Homocysteine is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Homocysteine is metabolised to either cysteine or to methionine. 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. Other less severe genetic defects which lead to moderately

elevated levels of homocysteine are also found. Drugs such as methotrexate, carbamazepine, phenytoin, nitrous oxide and penicillamine interfere with the Hcy metabolism and may give elevated levels of Hcy.

PERFORMANCE CHARACTERISTICS

PRECISION

	Average Hcy concentration	Within run precision	Total Precision
Low	6.1 µmol/L	8 %	10 %
Medium	10.5 µmol/L	7 %	9 %
High	20.6 µmol/L	8 %	10 %

Limit of quantification

The quantification limit of Homocysteine ELISA kit is 1.0 µmol/L with a CV < 20%.

Linearity of diluted plasma samples

If the homocysteine concentration of a sample exceeds the range of the calibration curve, the sample should be diluted with Reagent A and reanalysed.

The linearity was evaluated by diluting four high patient samples with varying amounts of Reagent A as diluent.

Linear regression analysis gave:

Slope: 0.98

Intercept: -0.4 µmol/L

Correlation coefficient r²: 0.99

Interfering Substances

The following endogenous substances were spiked into plasma samples and tested for interference by Homocysteine ELISA; Bilirubin, haemoglobin, lipids, protein, sodium fluoride and red blood cells.

The interference with this assay is < 10 % in the presence of: bilirubin (0.5 g/L), haemoglobin (10 g/L), triglycerides (10 g/L), red blood cells (5.0% v/v), protein (80 g/L) and sodium-fluoride (10 g/L).

Reference Range

The reference range should be determined by each laboratory to confirm the characteristics of the population being tested. As a point of reference, the following data may be used until the laboratory has analysed a sufficient number of samples to determine its own reference range.

The Hcy concentration in plasma or serum of healthy individuals varies with age, gender, geographical area and genetic factors. Scientific literature reports reference values for adult male and females between 5 and 15 µmol/L, men having higher values than women, and post menopausal women having higher homocysteine values than pre menopausal women. Hcy values will normally increase with age, giving a reference range among an elderly population (> 60 years) of 5 - 20 µmol/L. The median value of the homocysteine concentration among Scandinavians was 8.4 µmol/L, among Hispanics 8.9 µmol/L and among Americans 9.3 µmol/L.

The homocysteine reference range was established based on 95% confidence limits as 5 - 15 µmol/L for the Scandinavian population, 3.6 - 15.0 µmol/L for the American population and 2.9 - 16.0 µmol/L for the Hispanic population.

Measuring range:

The calibrator range is from 2.0 to 50.0 µmol/L.

Cross reactivity:

Adenosyl-L-methionine (SAM) (0.5 mmol/L) has a cross-reactivity of 16.3 % in a spiked sample. The cross reactivity with Homocysteine assay is < 1 % for Adenosine (5.0 mmol/L), Cystathionine (0.5 mmol/L), L-Cysteine (0.5 mmol/L), Gluthathione (100 mmol/L) and Thiolactone (0.5 mmol/L).

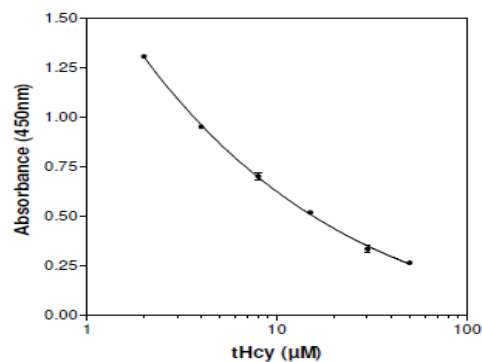
References:

Ueland PM. Homocysteine species as components of plasma redox thiol status. Clin.Chem. 1995;41:340-342.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation. Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Below is an example of a calibration curve using the four-parameter logistic curve fit.



PRINCIPLE OF THE TEST

Homocysteine is an enzyme immunoassay for the determination of homocysteine in blood. Protein bound homocysteine is reduced to free homocysteine and enzymatically converted to S-adenosyl-L-homocysteine (SAH) in a separate procedure prior to the immunoassay. The enzyme is specific for the L-form of homocysteine, which is the only form present in the blood. This enzyme immunoassay is based on competition between SAH in the sample and immobilised SAH bound to the walls of the microtitre plate for binding sites on a monoclonal anti-SAH antibody. After removal of unbound anti-SAH antibody, a secondary rabbit anti-mouse antibody labelled with the enzyme horse radish peroxidase (HRP) is added. The peroxidase activity is measured spectrophotometrically after addition of substrate, and the absorbance is inversely related to the concentration of homocysteine in the sample.

MATERIALS AND EQUIPMENT REQUIRED

Homocysteine controls, Adjustable micropipet (20-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, Vortex mixer, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International Homocysteine ELISA Kit is intended for *in vitro research* use only. This assay only measures L-homocysteine and not D-homocysteine. Reagent F contains mouse antibodies. Reagent G contains rabbit antibodies. The Negative, Positive and Calibrator controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions. Reagent D contains 0.15% merthiolate (< 0.074% mercury). Please handle and dispose of properly.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution)

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

As synthesis of Hcy continues in red blood cells after drawing, it is very important to prepare specimens as follows:

-Serum samples should be allowed to clot for no more than 30 minutes before centrifugation and separation of serum. Serum samples should be kept on ice prior to separation.

-EDTA-plasma samples must be centrifuged or put on ice immediately after drawing. EDTA-plasma samples may be kept on ice for up to 6 hours prior to separation by centrifugation.

Food consumption can affect circulating homocysteine levels. Protein rich meals give higher total homocysteine values and should be avoided late in the day before sampling. Plasma or serum samples may be stored for 12 weeks at 2 - 8 °C, for up to 3 weeks at room temperature (18 - 25 °C) and have been shown to be stable for at least 8 months if frozen at minus 20 °C. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. Haemoglobin, bilirubin or triglycerides do not interfere with the assay.

REAGENTS PREPARATIONS:

1) The **sample pre-treatment solution (SPS)** has to be made by mixing Reagent A, B and C. SPS has to be made just before the start of the assay. Volume needed per 10 samples: (No dead volume calculated) 4.5 mL Reagent A + 0.25 mL Reagent B + 0.25 mL Reagent C and Mix.

SPS solution is stable for one hour and has to be freshly made for each run.

2) The wash buffer solution should be diluted **1:10 with distilled water** before use. The prepared wash buffer is stable for 4 weeks when stored at room temperature (18-25 °C).

3) Reagent D and H are stored in dark bottles to avoid exposure to light.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. Store microplate sealed and desiccated in the clip bag provided. Shelf life of the unopened test kit is 18 months from day of production. Avoid exposure of the kit to temperatures exceeding 37 °C as this may denature the enzymes.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Label or mark the microtiter well strips to be used on the plate. We recommend to run the calibrators in duplicate. It is important that the microtitre strips are kept dry, i.e. in the sealed bag with drying capsules, and stored refrigerated. Equilibration for a minimum of two hours is required to reach room temperature (18 - 25 °C). Leave the strips in the bag during equilibration.

Note: Standards and controls are supplied ready to use. Sample pre-treatment procedure:

1. Dilute calibrators and samples / controls in plastic or glass tubes (25 µL calibrator/sample/control + 500 µL **SPS**) and Mix well. Incubate for 30 minutes at 37 °C. Cap the tubes or cover with parafilm during incubation. Proceed with step 2 before the samples have cooled.
2. Add 500 µL **Reagent D** and mix well. Incubate for 15 minutes at 18-25 °C.
3. Add 500 µL **Reagent E** and mix well. Incubate for 5 minutes at 18-25 °C.
Microtitre plate procedure:
4. Pipette **25 µL** diluted calibrator /sample / control from *step 3* into the wells of the SAH-coated microtiter strips.
5. Add 200 µL **Reagent F** to each well and Incubate for 30 min at 18-25 °C. Use the enclosed lid during all incubations.
6. Wash with diluted Wash buffer, 3 × 400 µL. (If manual washing is required, use 4 times 350 µL instead of 3 times 400 µL. After washing, empty the wells on paper towels)
7. Add 100 µL **Reagent G** to each well and Incubate 20 min at 18-25 °C.
8. Wash with diluted **Wash buffer**, 3 × 400 µL. (If manual washing is required, use 4 times 350 µL instead of 3 times 400 µL. After washing, empty the wells on paper towels)

9. Add 100 µL of **TMB** to each well and Incubate for 10 min at 18-25 °C.
10. Add 100 µL of **Stop solution** to each well.
11. Shake and read at 450 nm within 15 minutes. Automatic plate shaker is preferred to ensure proper mixing.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Interpretation of results

Results should be interpreted considering all other test results and the clinical status of the patient. We recommend that a four parameter logistic curve fit is used for preparing the calibration curve and calculation of unknown samples.

We recommend each laboratory to use an homocysteine control with known value. Homocysteine reference range was established based on 95 % confidence limit as 5 -15 µmol/L.

LIMITATIONS OF PROCEDURE

Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibody (HAMA). HAMA, present in serum or plasma specimens, may interfere in immunoassays which utilise mouse monoclonal antibodies. These specimens should not be assayed with Homocysteine Enzyme Immunoassay. Specimens from patients taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anti-convulsants or 6-azauridine triacetate, may have elevated levels of homocysteine due to metabolic interference with the homocysteine metabolism. This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually. The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.