Version 22-04/24

User's Manual

Homocysteine ELISA kit

REF **DEIABL3**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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

General Description

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.

Principles of Testing

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.

Reagents And Materials Provided

1. S-adenosyl-L-homocysteine coated microwell strips (12 × 8 wells), Ready-to-use, 1 Plate
2. S-adenosyl-L-homocysteine Standard A (2 µmol/L) in Assay buffer with preservative 1.5 ml, 1 bottle
3. S-adenosyl-L-homocysteine Standard B (4 µmol/L) in Assay buffer with preservative 1.5 ml, 1 bottle
4. S-adenosyl-L-homocysteine Standard C (8 µmol/L) in Assay buffer with preservative 1.5 ml, 1 bottle
5. S-adenosyl-L-homocysteine Standard D (15 µmol/L) in Assay buffer with preservative 1.5 ml, 1 bottle
6. S-adenosyl-L-homocysteine Standard E (30 µmol/L) in Assay buffer with preservative 1.5 ml, 1 bottle
7. S-adenosyl-L-homocysteine Standard F (50 µmol/L) in Assay buffer with preservative 1.5 ml, 1 bottle
8. Reagent A Assay buffer (Phosphate buffer, 0.09% NaN_3), 54 ml, Ready-to-use, 1 bottle
9. Reagent B Adenosine/DTT, 3.5 ml, 1 bottle

10. Reagent C SAH-Hydrolase, 3.5 ml, Ready-to-use, 1 bottle
11. Reagent D Enzyme inhibitor, 55 mL, Ready to use. 1 bottle (Dark bottle)
12. Reagent E Adenosine deaminase, 55 mL, Ready to use. 1 bottle (Red coloured)
13. Reagent F Monoclonal mouse-anti-S-adenosyl-Lhomocysteineantibody, 25 mL, Ready to use. 1 bottle
14. Reagent G Rabbit Anti-mouse-antibody enzyme conjugate, 15 mL, Ready to use. 1 bottle (Blue coloured)
15. Wash buffer (10×), 60 ml, dilute 1:10 with distilled water before use. 1 bottle
16. HRP Substrate Solution, 15 ml, 1 bottle
17. Stop solution, 20 ml, 1 bottle

Materials Required But Not Supplied

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.

Storage

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 dessicated in the clip bag provided. Shelf life of the unopended 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.

Specimen Collection And Preparation

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

1. 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.
2. 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.

Note:

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.

Reagent Preparation

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.
4. Standards and controls are supplied ready to use.

Assay 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.

Sample pretreatment 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 4oC. 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.

Calculation

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.

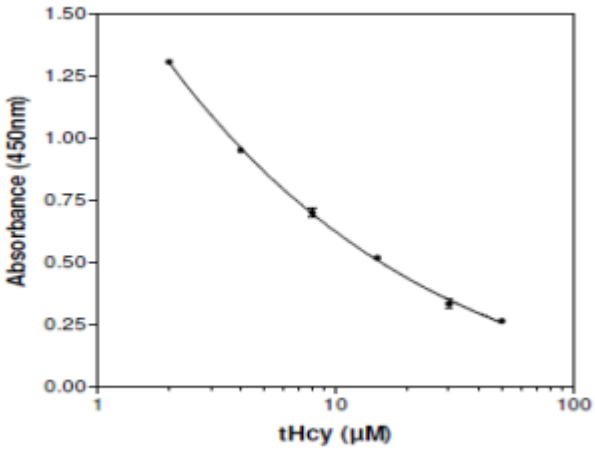
Recommended Lin-Log Plot

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.

Typical Standard Curve



Reference Values

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.

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 %

Detection Range

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

Detection Limit

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

Specificity

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).

Linearity

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 r^2: 0.99

Interferences

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).

Precautions

The CD 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.

Limitations

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.