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Inspiration for life science

Wel- Chem *Homocysteine 2 Reagent Enzymatic Assay Kit*

Configuration

The Wel-Chem Homocysteine 2 Reagent Enzymatic assay is provided in the following kit configurations:

Instrument	Catalog No.	Kit size(67ML)
Universal	HCY-145WB	R1: 1 x 52.8 mL R2: 1 x 14.2 mL Cal: 3 x 1 mL

Instrument	Catalog No.	Kit size(25ML)
Universal	HCY-245WB	R1: 1 x 19.68 mL R2: 1 x 5.32 mL Cal: 3 x 1 mL

Calibrator Sold Separately

Intended Use

The Wel-Chem Homocysteine 2 Reagent Enzymatic Assay 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 hyper homocysteinemia and homocystinuria. Weldon Homocysteine Enzymatic Assay 2-Reagent System is not intended for detecting or predicting deficiency of cobalamin or folate in patient samples by correlating with elevated total 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 intracellular 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.[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 tHcy levels are caused by four major factors, including: a) genetic deficiencies 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[4] and osteoporosis[5]. Guidelines for tHcy determination in clinical laboratories have recently been established.[6]

Assay Principle

The Weldon Homocysteine 2 Reagent Enzymatic 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 described in the literature. In this assay, oxidized Hcy is first 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 dehrogenase, 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 Hcy 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^+ (A_{340nm}).

Materials required but not provided

An analyzer capable of dispensing 2 reagents and measuring absorbance at 340 nm with temperature control (37° C).

Reagent Composition

Active Ingredients	Concentration
S-adenosylmethionine (SAM)	0.1 mM
NADH	0.2 mM
TCEP	0.5 mM
2-oxoglutarate	5.0 mM
Glutamate dehydrogenase	10 KU/L
SAH hydrolase	3.0 KU/L
Adenosine deaminase	5.0 KU/L
Hcy methyltransferase	5.0 KU/L

Reagent Preparation

The Weldon Homocysteine 2 Reagent Enzymatic assay reagents are ready-to-use liquid stable reagents. Calibrators and controls are ready-to-use stable liquids.

Reagent Stability and Storage

The Wel-Chem Homocysteine 2 Reagent Enzymatic assay reagents, calibrators, and controls should be stored at 2-8° C. **DO NOT FREEZE**. The reagents, calibrators, and controls are stable when stored as instructed until the expiration date on the label. Do not mix reagents of different lots.

Specimen Collection and Handling

Fresh serum, heparin plasma, or EDTA plasma can be used in 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 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, stable for several weeks at 0-8° C, and stable for several months or years at –20° C.[7]

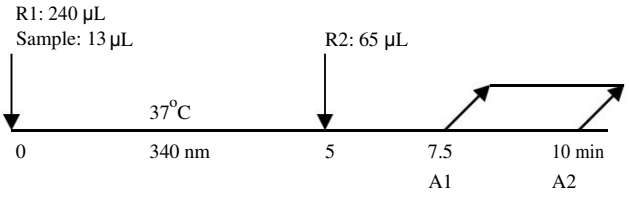
Precautions

The reagents are for *in vitro* diagnostic use only. **DO NOT IN-GEST** . Avoid contact with skin and eyes. Contains sodium azide, which may react with lead or copper plumbing to form explosive compounds. Flush drains with copious amounts of water when disposing of this reagent. Calibrators and controls are human serum based. Specimens containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395). Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

Assay Procedure

Reagent 1, Reagent 2, and Calibrators are packaged ready-to-use.

Before sampling, gently swirl the calibrator and control vial several times to ensure homogeneity. After each use, promptly replace the cap and return to 2-8° C storage.



Application sheets for use of the assay on other automated clinical chemistry analyzers are available upon request.

Calibration

For analyzers, use Calibrators 1-3 for calibration.
The calibration curve is stable for at least five days.

Quality Control

We recommend that each laboratory use Hcy controls to validate the performance of Hcy reagents. A set of normal and abnormal ranges of Hcy controls is available from Weldon. The range of acceptable control limits should be established by individual laboratories.

Results

Results are printed out in µmol/L. Note: Samples with values greater than50 µmole/L should be diluted 1:1 with water and rerun. Multiply results by 2.

Reference Range

In most of the U.S. clinical laboratories, 15 µmol/L is used as the cut-off value for normal level of Hcy for adults.[8-9] In Europe, 12 µmol/L is used as the cut- off value. However, each laboratory is recommended to establish a range of normal values for the population in their region.

Limitations

- The measuring range of the assay is from 3 to 50 µmol/L. Samples with Hcy values higher than 50 µmol/L should be diluted 1:1 with water.
- The reagent should be clear. It should be discarded if it becomes turbid or the initial absorbance is less than 0.5 at 340 nm (light path 0.6 cm).
- S-adenosylhomocysteine (SAH) will cause significant positive interference. However, SAH is either not detectable or at sub-nmole/L concentrations in normal plasma, and should not cause concern.
- 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.
- Addition of 3-deazaadenosine to inhibit Hcy production in red cells has been suggested. However, the WeldonHcy assay can not use samples containing 3-deazaadenosine since it inhibits one of the key enzymes used in the assay.

Performance Characteristics

Limit of Detection

To demonstrate the limit of detection (LOD) of the Weldon Homocysteine 2 Reagent Enzymatic Assay, Homocysteine zero calibrator was tested with 12 replicates. The LOD was defined as mean +3SD.

Zero Calibrator	
n	12
Mean	0.05
SD	0.117
Mean+3SD	0.40
LOD= 0.4 µM HCY	

Accuracy

Correlation studies were performed by testing 40 serum samples in comparison with an existing commercial Hcy assay method.

Linear regression gives a correlation coefficient r^2 value of 0.99, slope of 0.94 and y intercept of 1.05.

Precision

Precision studies were conducted according to the NCCLS EP-5 protocol with the following modifications. For within precision, four HCY serum samples containing 7.0, 12.0, 15.6, and 29.0 µM HCY were tested with HCY Enzymatic Assay on OLYMPUS AU400 with 20 replicates within one day. Within run imprecisions (CV%) for four levels of Hcy serum samples are 4.5% for 7 µM Hcy, 1.87% for 12 µM Hcy, 3.04% for 15.6 µM Hcy, and 2.4% for 29.0 µM Hcy. For inter run precision, four HCY serum samples containing 7.0, 12, 15.6 and 29.0 µM HCY were tested with 2 runs per day with triplicates over 5 days. Inter imprecision for three levels of Hcy controls are 5.87% for 7 µM Hcy, 4.88% for 12 µM Hcy, 5.51% for 15.6 µM Hcy, and 2.57% for 29.0 µM Hcy.

Linearity

The assay is linear up to 50 µmol/L.

Interference

An interference study was performed by testing a serum sample spiked with varied concentrations of endogenous substances. The following substances normally present in the serum produced less than 10% deviation when tested at the stated concentrations: 40 mg/dL Bilirubin, 1000 mg/dL Triglycerides, 500 mg/dL Hemoglobin, 40 mg/dL Bilirubin Conjugate, 10 mM Ascorbic Acid, and 100 µM** Cystathionine.

** The concentrations tested are about 5-10 times higher than the normal range of serum levels.

References

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