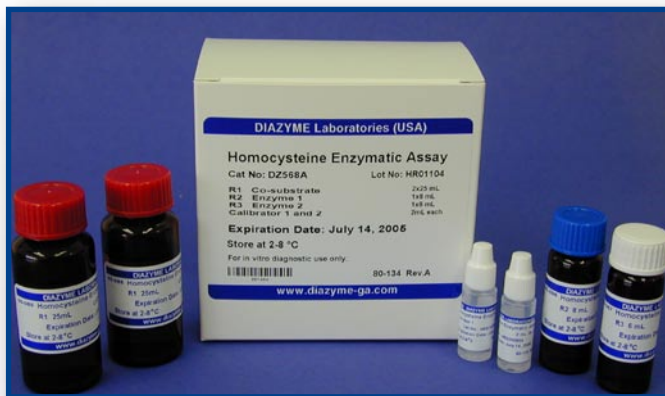


Homocysteine Assay

Enzyme Cycling



Accurate

- Excellent correlation to HPLC and Immunochemical methods

Precise

- Within-run and total precision have CV's of less than 5%

Efficient

- Diazyme's patented enzyme cycling method is fast with results in as little as ten (10) minutes enabling improved laboratory workflow and consolidation of multiple testing platforms

Reliable

- No "carry over" issues with iron or lipase reagents. Assay performance is unaffected by cystathionine (100µM), lipemia (2500 mg/dL), hemolytic samples (1200 mg/dL), Ascorbic Acid (10 mmol/L), Bilirubin (40mg/dL), Bilirubin Conjugate (40 mg/dL)

Convenient

- Dual stable liquid with a variety of instrument specific packaging options. As a Roche Channel Partner, the reagent is provided ready to use for the Roche COBAS INTEGRA® 400, 800, cobas 6000 and cobas c 501 systems in cobas c packs

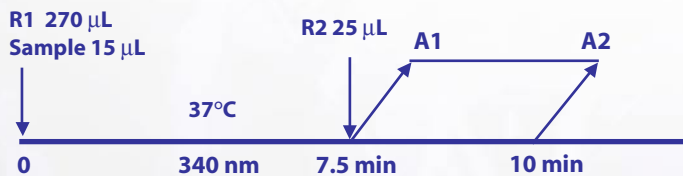
Homocysteine Assay

SUMMARY OF PERFORMANCE

Background

Total homocysteine (tHcy) represents the sum of all forms of Hcy including forms of oxidized, protein bound and free. Elevated tHcy has emerged as an important risk factor in the assessment of cardiovascular disease. Excess Hcy in the blood stream 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 the result of four major causes including: a) genetic deficiencies in enzymes involved in Hcy metabolism; b) nutritional deficiency in B vitamins such as B6, B12 and folate; c) renal failure for effective amino acid clearance; and d) drug interactions that interfere with Hcy metabolism. Elevated levels of tHcy are also linked with Alzheimer's disease and Osteoporosis.

Assay Method



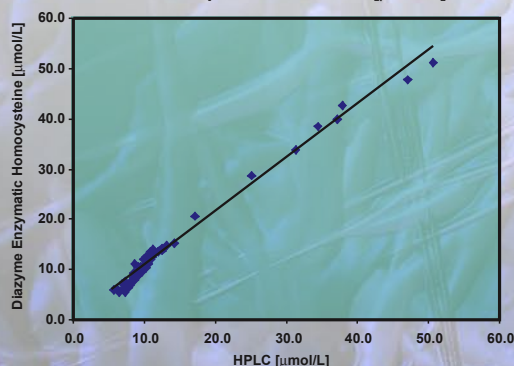
In this assay, oxidized Hcy is reduced to free Hcy which then reacts with S-adenosylmethionine (SAM) to form methionine and S-adenosylhomocysteine (SAH). SAH is assessed by coupled enzyme reactions wherein SAH is hydrolyzed into adenosine (Ado) and Hcy. The Hcy formed from the co-substrate SAM is cycled into the Hcy conversion reaction. This forms a co-substrate conversion product based enzyme cycling reaction system with significant amplification of detection signals. The Ado formed is 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⁺ (ΔA_{340nm}).

Performance

Accuracy

To demonstrate accuracy, the Diazyme Homocysteine Enzymatic Assay was tested with individual serum or plasma samples with comparison to an HPLC method. To ensure the concentrations of homocysteine were distributed across the reportable dynamic range, some homocysteine samples used for the study were spiked with stock solution of homocysteine to targeted concentrations. The Diazyme Homocysteine Enzymatic assay method (y) results generated on a Roche Hitachi 917™ instrument were compared with those obtained with a commercially available HPLC method (x). A total of 66 patient samples with values ranging from 5.7 to 51.2 µmol/L were analyzed. These studies yielded a correlation coefficient of 0.9935 with a linear regression of $y = 0.9181x + 0.355$.

Diazyme Enzymatic Homocysteine Comparisons to HPLC [µmol/L]



Precision

The within-run and between-run precision was evaluated according to the NCCLS EP5 protocol. In the study, three specimens were tested with 2 runs per day with duplicates over 20 working days. The results are summarized in the following table.

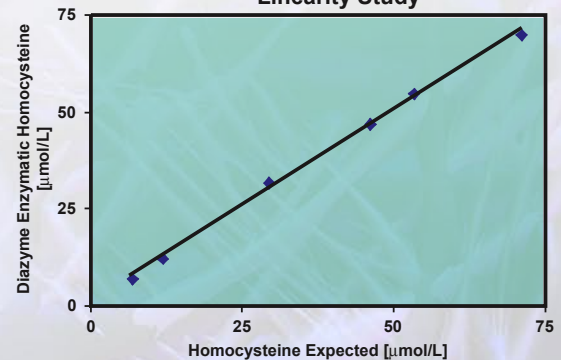
Within-run Precision	Level 1	Level 2	Level 3
Mean µmol/L	7.2	13.2	29.1
SD µmol/L	0.156	0.639	0.811
CV%	2.2	3.0	1.8

Between-run Precision	Level 1	Level 2	Level 3
Mean µmol/L	7.2	13.2	29.1
SD µmol/L	0.285	0.72	0.92
CV%	4.1	5.9	4.0

Linearity

Diazyme's Homocysteine Enzymatic Assay has a linear range from 0.3 to 50 µmol/L.

Diazyme Enzymatic Homocysteine Linearity Study



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: 500 µM NH₄Cl, 1 mM NaPi, 1 mM NaF, 2500 mg/dL Triglyceride, 20 mg/dL Bilirubin, 1200 mg/dL Hemoglobin, 0.5 mM Glutathione, 10 mM Ascorbic Acid, 1 mM L-Cysteine, 20 µM S-adenosylmethionine (SAM), 100 µM Adenosine, 100 µM Cystathionine.

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Available in a variety of convenient instrument specific packaging formats including Roche Hitachi, Olympus and Beckman. Companion products available for this product include control and calibrator sets.