



HOMOGENEOUS ENZYMIC/ENZYMATIC HOMOCYSTEINE 3 – PART REAGENT

INTENDED USE:

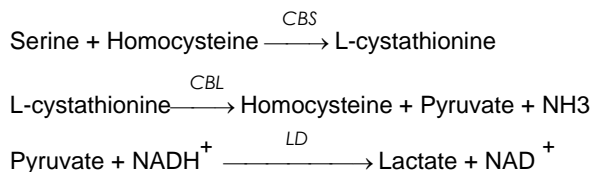
Catch Incorporated's Homogeneous Enzymic/ Enzymatic Homocysteine Reagent is intended for *in vitro* quantitative determination of total homocysteine in serum and plasma.

CLINICAL SIGNIFICANCE:

Homocysteine (HCY) is a by-product of protein metabolism, specifically the breakdown of the essential amino acid, methionine. It is not itself incorporated into proteins but is further broken down by enzymes into cysteine, or is converted back to methionine. In small amounts, HCY is not harmful to the body or blood vessels, but when excess amounts accumulate in the blood stream, arterial vessels may be damaged and the resulting inflammation may eventually cause blockage of blood to the heart. Clinical studies began in 1969, when Dr. Kilmer S. McCully reported that children born with a genetic error of metabolism that causes their homocysteine levels to be significantly elevated (homocystinuria), died with advanced artery disease at a very young age⁽¹⁾. More recent studies are yielding evidence that elevated blood levels of homocysteine have a predictive value for risk of coronary artery disease similar to that of elevated cholesterol levels^(2, 3).

METHOD SUMMARY:

Oxidized forms of homocysteine are reduced to free homocysteine which then reacts with serine catalyzed by cystathionine β -synthase (CBS) to form L-cystathionine. Cystathionine is cycled back to homocysteine by cystathionine β -lyase (CBL) forming, in the process, pyruvate and ammonia. This cycling sequence increases the sensitivity of the method by about ten-fold. Pyruvate is detected using lactate dehydrogenase with NADH. The rate of conversion of NADH to NAD is directly proportional to the amount of homocysteine in the sample or calibrator.



REAGENT COMPOSITION

Active Ingredients	Concentration
Lactate Dehydrogenase	> 35 KU/L
Serine	1.3 mmol/L
NADH	0.56 mmol/L
Cystathionine β -Synthase (Microbial)	> 20 KU/L
Cystathionine β -Lyase (Microbial)	> 10 KU/L
Buffers and Stabilizers	

REAGENT PREPARATION:

R1, R2 and R3 are packaged ready to use.

CALIBRATOR PREPARATION AND USE:

The calibrators are prepared gravimetrically and supplied as a 2-level set ready to use. Values are printed on the labels.

WARNING:

Specimens from patients taking methotrexate, carbamazepine, phenytoin, nitrous oxide, 6-azauridine triacetate or S-Adenosyl methionine may have elevated levels of homocysteine due to their effect on the metabolic pathway.

This reagent is for *in vitro* diagnostic use only. **DO NOT INGEST.** 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. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please call Catch Incorporated at:

1 (866) 719-6099

STABILITY AND STORAGE:

The Homocysteine Reagents should be stored refrigerated at 2-8 °C. **DO NOT FREEZE.** The reagents are stable when stored as supplied until the expiration date on the label. The reagent is stable on board for 30 days. Do not mix different lots of reagent.

SPECIMEN COLLECTION AND HANDLING:

Fresh EDTA or heparinized plasma free of hemolysis or turbidity is the recommended specimen for measurement of homocysteine. Serum collected in serum separator tubes may also be used. However, it is not recommended to use individual patient results from serum, heparinized plasma or EDTA plasma interchangeably. All samples must be placed on ice immediately after collection and should be centrifuged and separated from cells as soon as possible (within one hour). Severely lipemic specimens (4+) are unsatisfactory. Samples should be stored tightly capped at 2-8 °C for up to 48 hours, or frozen at -20 °C if testing is delayed⁽⁵⁾.

PROCEDURE:

Materials provided:

The Catch Homocysteine Reagent kit,

Catalog Number. 3-501

R1:	1 x 36 mL
R2:	1 x 15 mL
R3:	1 x 7 mL
Calibrator:	2 x 3 mL

*** The number of tests that can be obtained from this kit is dependent on the analyzer used.**

Materials Required but not provided:

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

Saline (0.85%) for dilutions

Test Conditions:

Wavelength	340 or 340/380 nm
Pathlength	0.5 – 0.7 cm
Temperature:	37 °C
Sample/Reagent1/ Reagent 2	
/Reagent 3	1/12/1.4/1.2
Reaction 1:	Add Sample, R1, R2, incubate 2 – 4 min.
Reaction 2:	Add R3, Measure rate (decrease) over 2 – 5 min

Various instrument applications are available at www.catchinc.net or 1-866-719-6099

RESULTS:

Results are printed out by the Hitachi 911 in $\mu\text{mol/L}$.

QUALITY CONTROL:

Assayed control material with values for Homocysteine in both the normal and abnormal ranges should be tested to validate reagent performance. The range of acceptable control limits should be established by individual laboratories. Follow federal, state and local requirements for quality control.

LIMITATIONS:

- Calibration stability is 5 days, but can vary with instrument systems and laboratory conditions.
- Linearity extends to 50 $\mu\text{mol/L}$. Samples with values greater 50 $\mu\text{mol/L}$ should be diluted 1:3 in 0.85% saline or zero calibrator and the result multiplied by three (x3).
- The reagent should be clear. Discard if turbid.
- The mean cystathionine (Cys) level in plasma is 100 times less than that of homocysteine (10 $\mu\text{mol/L}$). Therefore, cys has no effect on the assay of homocysteine in the general population. In end stage renal disease (ESRD), while the cys level increases (mean = 2.0 $\mu\text{mol/L}$) the mean homocysteine value also increases (mean = 30

$\mu\text{mol/L}$). Therefore, cys has no clinically significant effect on the assay of homocysteine in typical ESRD.

- Hydroxylamine, if present in the iron reagents used on the analyzer, could interfere through probe or cuvette carryover. For further information concerning elimination of this effect contact technical service.

EXPECTED VALUES:

A study was conducted using samples from 131 adults (ages 18-86) who were seemingly healthy. Plasma (EDTA) samples were assayed for total homocysteine by the Catch Homocysteine Assay and the reference range for homocysteine by this method is:

Adult 4.0-15.4 $\mu\text{mol/L}$

These values are to be considered as guidelines. Each laboratory is encouraged to establish a range of normal values for the population they serve.

PERFORMANCE CHARACTERISTICS:

(Established on the Hitachi 911)

Accuracy: Correlation studies were performed with fresh adult plasma. All samples were analyzed using the Catch Homocysteine Assay and the Abbott FPIA assay. The data obtained gave the following statistical values:

COMPARISON METHOD	
Abbott FPIA	Catch HCY
NUMBER OF SAMPLES	55
SLOPE OF REGRESSION LINE	1.047
Y-INTERCEPT	-0.38
CORRELATION COEFFICIENT	0.991
RANGE ($\mu\text{mol/L}$)	5.8-37.6

Precision: Precision studies were conducted utilizing the Catch Homocysteine reagent according to the NCCLS EP-5 protocol. Within-run and between-run precision studies were done on human plasma EDTA pools at low, mid and elevated levels. Results are summarized below:

	Within Run			Between Run		
	Low	Mid	Elev	Low	Mid	Elev.
N	80	80	80	80	80	80
Mean ($\mu\text{mol/L}$)	6.6	15.5	24.6	6.6	15.5	24.6
SD	0.22	0.28	0.38	0.24	0.19	0.23
CV%	3.7	1.8	1.5	3.9	1.2	0.9

Sensitivity: The sensitivity of the Catch Homocysteine assay was found to be 0.54 $\mu\text{mol/L}$.

Specificity: Bilirubin to 20 mg/dL and lipemia to 1000 mg/dL do not interfere significantly with this method (< 5%). Hemoglobin to 1000 mg/dL shows <10% interference. Glutathione, Methionine, Cysteine, and pyruvate at levels of 20, 800, 100, and 1000 $\mu\text{mol/L}$ respectively cause < 5% variation. Refer to references ^(4, 5) for possible interferences caused by drugs, disease or preanalytical variables.

Carryover: Carryover studies show that carryover is less than the sensitivity of the assay.

BIBLIOGRAPHY

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3. Cramer DA. Homocysteine vs cholesterol--competing views, or a unifying explanation of arteriosclerotic cardiovascular disease? *Lab. Med.* 1998; 29 (7): 410-417.
4. Young, DS. Effects of drugs on clinical laboratory tests, 5th ed., AACCC Press, Washington, DC, 2000, p 3-441.
5. Ueland PM, Refsum H, Stabler SP, *et al*, "Total Homocysteine in Plasma or Serum: Methods and Clinical Applications," *Clin Chem* 1993, 39:1764-79.

Manufactured by

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