3-Reagent Homocysteine Assay for SYNCHRON UniCel DxC

REF B08175

(Distributed by BECKMAN COULTER, for professional use only, on the BECKMAN COULTER SYNCHRON UniCel System)

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IVD

Σ 100

ENGLISH: INTENDED USE

The 3-Reagent Homocysteine Assay for SYNCHRON UniCel DxC System is intended for *in vitro* quantitative determination of total homocysteine in human serum and plasma. The device can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

WARNING: Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine. Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azauridine triacetate may have elevated levels of homocysteine due to their effect on the pathway. Refer to the LIMITATIONS FOR USE section in this assay package insert.

SUMMARY AND EXPLANATION OF TEST

Homocysteine (HCY) is a thiol-containing amino acid produced by the intracellular demethylation of methionine. Homocysteine is exported into plasma where it circulates, mostly in its oxidized form, bound to plasma proteins as a protein-HCY mixed disulfide with albumin (protein-SS-HCY).[1-5] Smaller amounts of reduced homocysteine and the disulfide homocystine (HCY-SS-HCY) are present. Total homocysteine (tHCY) represents the sum of all the HCY species found in serum or plasma (free plus protein bound). Homocysteine is metabolized to either cysteine or methionine. In the vitamin B6 trans-sulphuration pathway, homocysteine is irreversibly catabolised to cysteine. A major part of homocysteine is remethylated to methionine, mainly by the folate and cobalamin-dependent enzyme methionine synthase. Homocysteine accumulates and is excreted into blood when these reactions are impaired.[3,5] Severely elevated concentrates of total homocysteine 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.[2,6] Other less severe genetic defects which lead to moderately elevated levels of total homocysteine are also found.[7-9]

Epidemiological studies have investigated the relationship between elevated homocysteine levels and cardiovascular disease (CVD). A meta-analysis of 27 of these studies, including more than 4000 patients, estimated that a 5 µmol/L increase in total homocysteine was associated with an odds ratio for coronary artery disease (CAD) of 1.6 (95% confidence interval [CI], 1.4 to 1.7 for men and 1.8 (95% CI 1.3 to 1.9) for women; the odds ratio for cerebrovascular disease was 1.5 (95% CI 1.3 to 1.9). The risk associated with a 5 µmol/L increase in total homocysteine was the same as that associated with 0.5 mmol/L (20 mg/dL) increase in cholesterol. Peripheral arterial disease also showed a strong association.[10]

Hyperhomocysteinemia, elevated levels of homocysteine, can be associated with an increased risk of CVD. There have also been many published reports of prospective studies on the relationship between hyperhomocysteinemia and risk of CVD in men and women who were initially healthy. End points were based on a cardiovascular event such as acute myocardial infarction, stroke, CAD, or mortality. The results of eleven of these nested case-control studies reviewed by Cattaneo[11] were equivocal where five of the studies support the association with risk and six do not. More recently homocysteine levels were determined in a prospective study of post-menopausal women who participated in the Women's Health Study. Specimens from 122 women, who subsequently developed cardiovascular events, were tested for homocysteine and compared to a control group of 244 women who were matched for age and smoking status. The women in the control group remained free of disease during the three year follow-up period. The results demonstrated that post-menopausal women who developed cardiovascular events had significantly higher baseline homocysteine levels. Those with levels in the highest quartile had a two-fold increase in risk of any cardiovascular event. Elevated baseline homocysteine levels were shown to be an independent risk factor.[12] Also, homocysteine levels were determined in 1933 elderly men and women for the Framingham Heart Study cohort and demonstrated that elevated levels of homocysteine are independently associated with increased rates of all-cause and CVD mortality.[13]

Patients with chronic renal disease experience an excess morbidity and mortality due to arteriosclerotic CVD. Elevated concentration of homocysteine is a frequently observed finding in the blood of these patients. Although such patients lack some of the vitamins involved in the metabolism of homocysteine, the elevated HCY levels are mainly due to impaired HCY removal from the blood by the Kidneys.[14,15]

Drugs such as methotrexate, carbamazepine, phenytoin, nitrous oxide, and 6-azauridine triacetate interfere with HCY metabolism and may give elevate levels of HCY.[16]

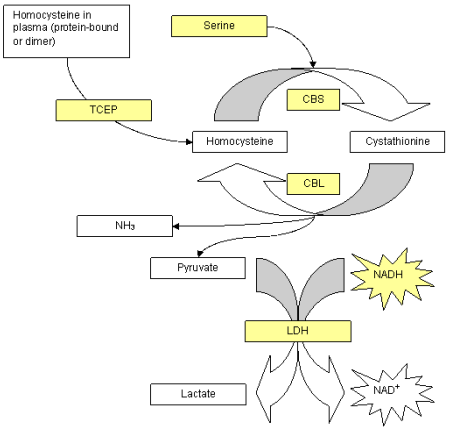
PRINCIPLE OF THE ASSAY

Bound or dimerised homocysteine (oxidised form) is reduced to free homocysteine, which then reacts with serine catalysed by cystathionine beta-synthase (CBS) to form cystathionine. Cystathionine in turn is broken down by cystathionine beta-lyase (CBL) to form homocysteine, pyruvate and ammonia. Pyruvate is then converted by lactate dehydrogenase (LDH) to lactate with nicotinamide adenine dinucleotide (NADH) as coenzyme. The rate of NADH conversion to NAD^+ is directly proportional to the concentration of homocysteine (Δ A340 nm).

Reduction: Dimerised homocysteine, mixed disulfide, and protein-bound forms of HCY in the sample are reduced to form free HCY by the use of tris [2-carboxyethyl] phosphine (TCEP).

HCY-SS-HCY (dimerised homocysteine)
R1-SS-HCY (R1 = thiol residue)
Protein-SS-HCY

TCEP → HCY



Enzymatic Conversion: Free HCY is converted to cystathionine by the use of cystathionine beta-synthase and excess serine. The cystathionine is then broken down to homocysteine, pyruvate and ammonia.

Pyruvate is converted to lactate via lactate dehydrogenase with NADH as coenzyme.

The rate of NADH conversion to NAD^+ (Δ A340 nm) is directly proportional to the concentration of homocysteine.

ADDITIONAL INFORMATION

Since Beckman Coulter does not manufacture the reagent or perform quality control or other tests on individual lots, Beckman Coulter cannot be responsible for the quality of the data obtained which is caused by performance of the reagent, any variation between lots of reagents, or protocol changes by the manufacturer.

TECHNICAL SUPPORT

- For Technical Support, please contact your local Beckman Coulter Representative.
- Please notify your Beckman Coulter Clinical Support Center if this product is received damaged.
- For instructions for use (including translations), please visit – www.homocysteine.org.uk/BCI

ORDERING INFORMATION AND KIT COMPONENTS

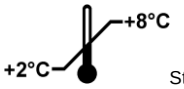
The following codes can be used to reorder materials from your local Beckman Coulter Representative;

Product Code	Configuration	Description	Composition	Hazard
B08175	1 x SYNCHRON® Assay Cartridge	REAG 1 - 36mL in Chamber A	NADH (0.45 g/L), Serine (0.108 g/L), Trizma Base 1-10%, Trizma Hydrochloride 1-10%, Sodium Azide < 1%. **Ready-to-use**	
		REAG 2 - 15mL in Chamber B	Reductant (TCEP:3.0 g/L) **Ready-to-use**	
		REAG 3 - 5mL in Chamber C	Cycling Enzymes CBS (0.748 KU/L) and CBL (16.4 KU/L), LDH (21.2 KU/L) Sodium Azide < 1%. **Ready-to-use**	
	1 x 3.0 mL in Opaque Vial (Blue Cap)	CAL 0µM	Aqueous homocysteine blank (0 µmol/L). **Ready-to-use**	
	1 x 3.0 mL in Opaque Vial (Red Cap)	CAL 28µM	Aqueous homocysteine solution (28 µmol/L). **Ready-to-use**	

The calibrators are prepared gravimetrically and are traceable to NIST SRM 1955, confirmed by a designated measurement procedure (HPLC). The values assigned are printed on the labels (0 µmol/L and 28 µmol/L).

A Homocysteine Control Kit (**Product Code - B08177**) containing low, medium and high controls is also available from Beckman Coulter for use with the 3-Reagent Homocysteine Assay for SYNCHRON UniCel DxC System.

STORAGE AND SHIPPING OF REAGENTS

1. 

Store kit components at 2-8˚C and use until the expiry date on the labels. Do not use expired reagents.
2. Please notify your Beckman Coulter Technical Support Center if this product is received damaged.
3. Reagents may be used on multiple occasions until the expiry date on the labels. Reagents **must** be returned to 2-8°C storage between use.
4. Do not mix different reagent kit lot numbers.
5. **DO NOT FREEZE REAGENTS**.
6. Do not expose Reagent material to light.
7. Avoid contamination of reagents. Use a new disposable pipette tip for each reagent or sample manipulation.
8. On-board instrument storage. The reagents can be stored for 30 days on-board the SYNCHRON UniCel DxC System.
9. The reagents should be clear of particulate material. They should be discarded if they become turbid.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only

1. Adhere strictly to the instructions in this leaflet, particularly for handling and storage conditions.
2. Reagent 1 and Reagent 3 contain sodium azide which can react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large quantities of water to prevent azide build-up.
3. Material safety data sheets for all hazardous components contained in this kit are available upon request from the product manufacturer, Axis-Shield Diagnostics Ltd.

EUH032: Contact with acids liberates very toxic gas.

Caution: Federal law restricts this device to sale by or on the order of a physician.

SPECIMEN COLLECTION AND HANDLING

1. Serum (collected in serum or serum separator tubes) and plasma (collected in potassium EDTA or lithium heparin tubes) may be used for the measurement of homocysteine.
   However, it is not recommended to use individual patient results from serum, heparinized plasma and EDTA plasma interchangeably.[26] Additionally matrix differences between serum and serum separator tubes and plasma tubes have been reported.[18]
   To minimize increases in homocysteine concentration from synthesis by red blood cells, process specimens as follows:
- Place all specimens (serum and plasma) on ice after collection and prior to processing. Serum may clot more slowly and the volume may be reduced.[16]
- All specimens may be kept on ice for up to 6 hours prior to separation by centrifugation.[16]
- Separate red blood cells from serum or plasma by centrifugation and transfer to a sample cup or other clean container.
  Note: Specimens not placed on ice immediately may exhibit a 10-20% increase in homocysteine concentration.[17]
2. If the assay will be performed within 2 weeks after collection, the specimen should be stored at 2-8˚C. If the testing will be delayed more than 2 weeks, the specimen should be stored frozen at -20˚C or colder. Specimens have been shown to be stable at -20˚C for 8 months.[16,18]
3. It is the responsibility of the operator to verify the correct specimen type(s) is (are) used in the 3-Reagent Homocysteine Assay for SYNCHRON UniCel DxC System.
4. Inspect all samples (specimens, calibrators and controls) for bubbles. Remove bubbles prior to analysis.
5. Specimens containing particulate matter (fibrin, red blood cells, or other matter) and visibly lipemic specimens should not be used with the assay. Results from these specimens may be inaccurate.
6. Mix specimens **thoroughly** after thawing by low speed vortexing or by gentle inversion to ensure consistency in results. Avoid repeated freezing and thawing. Specimens showing particulate matter, erythrocytes, or turbidity should be centrifuged before testing.
7. On-board instrument storage. EDTA plasma samples can be stored for 1.5 hours on-board the UniCel® DxC 600. The other recommended sample tubes for use with the assay have not been tested.

RESULTS

Results are reported in µmol/L.

EXPECTED VALUES

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 specimens 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 woman having higher homocysteine values than pre-menopausal women.[16,19,20] HCY values will normally increase with age, giving a reference range among an elderly population (> 60 years) of 5-20 µmol/L.[21] In countries with folic acid fortification programmes, reduced levels of HCY may be observed.[22,23]

Measurable Range: The measurable range of the 3-Reagent Enzymatic Homocysteine Assay for SYNCHRON UniCel DxC System is 1-50 µmol/L.

LIMITATIONS OF USE

1. The linear range of the 3-Reagent Homocysteine Assay on SYNCHRON UniCel DxC System when run as directed is 1-50 µmol/L. Specimens > 50 µmol/L should be diluted 1 part specimen to 2 parts Cal 0 µmol/L or 1 part specimen to 9 parts Cal 0 µmol/L as appropriate.
2. The Reagents should be clear. Discard if turbid.
3. Cystathionine is measured with homocysteine, but in the general population the cystathionine level (0.065 to 0.3 µmol/L) has a negligible effect. In very rare cases, end stage renal disease and patients with severe metabolic disturbances, cystathionine levels may rise dramatically and in severe cases cause greater than 20% interference.[24,25]
4. Carbamazepine, methotrexate, phenytoin, nitrous oxide, or 6-azauridine triacetate may affect the homocysteine concentration.[16]
5. Note: Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine. Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azauridine triacetate, may have elevated levels of homocysteine due to their effect on the pathway.
6. Specimens containing particulate matter (fibrin, red blood cells, or other matter) and visibly lipemic specimens should not be used with the assay. Results from these specimens may be inaccurate.

PERFORMANCE DATA

BASED ON MEASUREMENTS GENERATED ON SYNCHRON UniCel DxC 600

Accuracy

A correlation study was performed with the Axis-Shield Liquid Stable (LS) 2 Part Homocysteine Reagent Assay and the 3-Reagent Homocysteine Assay for SYNCHRON UniCel DxC with plasma specimens from 50 apparently healthy donors. The specimens were analysed using the Axis-Shield Liquid Stable (LS) 2 Part Homocysteine Reagent Assay on the Beckman Coulter AU 400 and the 3-Reagent Homocysteine Assay on the and UniCel DxC 600 instruments according to the CLSI (formally NCCLS) document EP9-A2.[27] All results are described using a 95% confidence Interval. Specimens results ranged from;

Axis-Shield Liquid Stable (LS) 2 Part Homocysteine Reagent Assay on Beckman Coulter AU 400 – Results ranging from 5.8 to 45.9 µmol/L.
3-Reagent Homocysteine Assay on UniCel DxC 600 – Results ranging from 6.7 to 46.1 µmol/L.

The data obtained gave the following statistical values:

Comparison Method	Beckman Coulter AU400 ***v.*** ***SYNCHRON UniCel DxC 600***
Number of specimens	50
Slope of regression line	0.99
Y-Intercept	0.74
Correlation coefficient	0.994

Precision

Studies on the SYNCHRON UniCel DxC 600 were performed with guidance from the CLSI (formally NCCLS) Document EP5-A2.[28] For each instrument three HCY controls and three human plasma samples were assayed using two lot of reagents, in replicates of two, at two separate times per day for 20 days on one instrument (n=80). Results are summarised below:

SYNCHRON UniCel DxC 600

Sample	Reagent Lot	Mean	Within-Run		Between-Run		Total	
			SD	%CV	SD	%CV	SD	%CV
Low Control	1	6.15	0.36	5.9	0.00	0.0	0.49	8.0
	2	6.37	0.30	4.7	0.00	0.0	0.40	6.3
Medium Control	1	11.65	0.49	4.2	0.36	3.1	0.63	5.4
	2	11.90	0.33	2.8	0.21	1.8	0.62	5.2
High Control	1	24.13	0.64	2.6	0.43	1.8	1.09	4.5
	2	24.37	0.63	2.6	0.59	2.4	1.13	4.6
Sample P1	1	7.43	0.47	6.3	0.13	1.7	0.53	7.2
	2	7.63	0.27	3.5	0.11	1.4	0.48	6.3
Sample P2	1	33.20	0.85	2.6	0.62	1.9	1.42	4.3
	2	33.58	0.79	2.4	0.65	1.9	1.83	5.4
Sample P3	1	45.38	1.08	2.4	1.27	2.8	1.92	4.2
	2	45.61	0.96	2.1	0.62	1.4	2.44	5.4

Dilution Linearity

The dilution linearity of the 3-Reagent Homocysteine Assay on SYNCHRON UniCel DxC System gives a % recovery range of 100% ± 10% for all samples across the range of the assay. Samples > 50 µmol/L exhibit mean recovery of 100% ± 14% of the expected result when diluted into the assay range.

Limit of Detection

The limit of detection (LOD) of the 3-Reagent Homocysteine Assay on SYNCHRON UniCel DxC System according to the CLSI (formally NCCLS) Document EP17-A[29] was found to be 0.89 µmol/L.

Analytical Specificity

The analytical specificity of the 3-Reagent Homocysteine Assay on SYNCHRON UniCel DxC System assessed according to guidance in the CLSI Document EP7-A2[30] for the interfering substances listed in the table below:

Interfering Substance	*Interfering Substance Concentration*	*% Interference*
Bilirubin	20 mg/dL	≤ ±10
Haemoglobin	500 mg/dL	≤ ±10
Triglyceride	1000 mg/dL	≤ ±10
Glutathione	1000 µmol/L	≤ ±10
Methionine	800 µmol/L	≤ ±10
L-Cysteine	200 µmol/L	≤ ±10
Pyruvate	1250 µmol/L	≤ ±10
Total Protein	120 mg/mL	≤ ±10

None of these substances interfered significantly in the assay.
Refer to Reference 16 in the references section of this pack leaflet for possible interferences caused by drugs, disease or preanalytical variables.

Probe/Cuvette Carryover

Carryover studies on the SYNCHRON LX 20 Pro show that Probe/Cuvette carryover of hydroxylamine, present in Beckman Coulter® Iron (FE) reagent, is ≤10% at HCY levels 25-30 µmol/L. Equivalency between the SYNCHRON LX and UniCel Systems has been established.

Sample Carryover

Sample carryover studies on the SYNCHRON UniCel DxC System show that carryover is less than the limit of detection of the assay.

On-board Reagent Stability

The reagents are stable on-board the SYNCHRON UniCel DxC System for 30 days.

Calibration Stability

The calibration curve on the SYNCHRON UniCel DxC System is stable for 14 days.

Specimen Types

The specimen collection tubes verified to be used with the 3-Reagent Homocysteine on SYNCHRON UniCel DxC System are EDTA and lithium heparin plasma tubes, serum and Serum Separator tubes. Other specimen collection tubes have not been tested.

However, it is not recommended to use individual patient results from serum, heparinized plasma and EDTA plasma interchangeably.[26] Additionally matrix differences between serum, Serum Separator tubes and plasma tubes have been reported.[18]

HCTX ASSAY PROTOCOL – SYNCHRON UniCel DxC 600/800

Ensure that the assay parameters exactly match those listed below.

ASSAY NAME: HCTX

CHEMISTRY PARAMETERS				
Reaction Type:	Rate 1	Calulation Factor:		1.000
Units:	µmol/L	No. of Calibrators:		2
Precision:	X.XX	Setpoints:	1	0.000
Reaction Direction:	Negative		2	28.000
Math Model:	Linear	Cal Time Limit:		336 hours
Primary Wavelength:	340			
Secondary Wavelength:	380			

Processing Parameters	**First Inject**	**Second Inject**	**Third Inject**
Component	A	B	C
Dispense Volume	185 µL	70 µL	38 µL
Inject Time		-180 sec	550 sec
Sample Volumes	25 µL		
	Blank	**Reaction 1**	**Reaction 2**
Start Read	-50 sec	600 sec	
End Read	-10 sec	720 sec	
	Lower Limit	**Upper Limit**	
Usable Result Range	1.000	50.000	

Error Detection Limits	**Blank**	**Reaction 1**	**Reaction 2**
ABS Low Limit	-1.500	-1.500	-1.500
ABS High Limit	2.200	2.200	2.200
Rate Low Limit	2.200	2.200	-1.500
Rate High Limit	-1.500	-1.500	2.200
Mean Deviation	2.200	2.200	2.200
Initial Rate	-99.999		
Delta ABS	2.200		
Multipoint Span (1-2)	-0.001		

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29. National Committee for Clinical Laboratory Standards. *Protocols for the Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.* NCCLS Document EP17-A. Wayne, PA: NCCLS, 2004.
30. Clinical Laboratory Standards Institute. *Interference Testing in Clinical Chemistry; Approved Guideline–Second Edition*. CLSI Document EP7-A2. Wayne, PA: CLSI, 2005.

IVD	*In Vitro* Diagnostic Medical Device	+2°C +8°C	Store at 2-8ºC
REF	Product code		Manufactured by
LOT	Lot number		Store in the dark
Σ 100	100 tests	REAG 1	Reagent 1, 2, 3
	Consult Instructions For Use	CAL	Calibrator 0 µmol/L , Calibrator 28 µmol/L
	Use by		Axis-Shield Diagnostics Ltd., The Technology Park, Dundee, DD2 1XA, UK *Tel:* +44 (0) 1382 422000 *Fax:* +44 (0) 1382 422088
Rx Only	Prescription Use Only		

Ver: 2019/09 RPBL1054/R6

CE

EC REP

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