Axis-Shield Liquid Stable (LS) 2-Part Homocysteine Assay

REF B08176

(Distributed by BECKMAN COULTER, for professional use only, on the BECKMAN COULTER AU platforms (AU400, AU480, AU680, AU5800, DxC 500 AU and DxC 700 AU))

Axis-Shield Diagnostics Ltd.
The Technology Park
Dundee DD2 1XA
United Kingdom
Tel: +44 (0) 1382 422000
Fax: +44 (0) 1382 422088

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IVD

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ENGLISH:

INTENDED USE

The Liquid Stable (LS) 2-Part Homocysteine Reagent 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. For Professional Use Only.

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

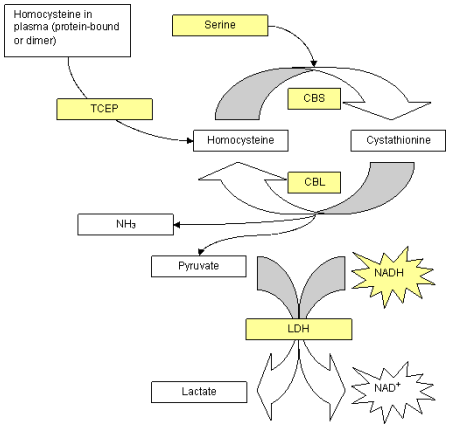
Bound or dimerised homocysteine (oxidised form) is reduced to free homocysteine, which then reacts with serine catalysed by cystathionine beta-synthase (CBS) to form cystathionine. Cystathionine in turn is broken down by cystathionine beta-lyase (CBL) to form homocysteine, pyruvate and ammonia. Pyruvate 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 (D 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.
- For Shipping Damage - please notify your Beckman Coulter Clinical Support Center if this product is received damaged.
- For instructions for use (including translations and cross contamination avoidance parameters), please visit – www.homocysteine.org.uk/BCI

ORDERING INFORMATION AND KIT COMPONENTS

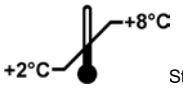
The following codes can be used to reorder materials from your local Beckman Coulter Representative;

Product Code	Description	Composition	Hazard
B08176	REAG 1 - 1 x 30 mL Colourless, odourless liquid	NADH (0.47 mM), LDH (38 KU/L), Serine (0.76 mM), Trizma Base 1-10%, Trizma Hydrochloride 1-10%, Sodium Azide < 1%. Reductant (TCEP:2.9 mM) **Ready-to-use**	
	REAG 2 - 1 x 5mL Pale yellow odourless liquid	Cycling Enzymes CBS (0.748 KU/L) and CBL (16.4 KU/L) Sodium Azide < 1%. **Ready-to-use**	
	CAL 0µM - 1 x 3.0 mL, (Blue Cap), Colourless odourless liquid	Aqueous homocysteine blank (0 µmol/L). **Ready-to-use**	
	CAL 28µM - 1 x 3.0 mL, (Red Cap), Colourless odourless liquid	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 Liquid Stable (LS) 2-part Homocysteine Reagent.

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 on all AU platforms (AU400, AU480, AU680, AU5800, DxC 500 AU and DxC 700 AU).
9. The reagents should be clear of particulate material. They should be discarded if they become turbid.

ASSAY PROCEDURE

1. Programme instrument using appropriate instrument protocols.
2. Load reagents and samples onto the instrument as instructed.
3. Run assay.

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 2 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.

Caution: For US applicable product, Federal law restricts this device to sale by or on the order of a physician.

Product Identifier: FHRW110	**Trade Name**	REAG 1
	Hazardous Substance	SODIUM AZIDE (EINECS: 247-852-1, CAS: 26628-22-8) ETHANOL (CAS: 64-17-5)
Classification		Flam. Liq. 3 H226 Flammable liquid and vapour
Hazard Pictogram		
Signal Word		WARNING
Hazard Statement		EUH032: Contact with acids liberates very toxic gas. H226 Flammable liquid and vapour.
Precautionary Statements		
Prevention		P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 Keep container tightly closed. P240 Ground and bond container and receiving equipment. P241 Use explosion-proof [electrical/ventilating/lighting] equipment. P242 Use non-sparking tools. P243 Take action to prevent static discharges. P273 Avoid release to the environment. P280 Wear protective gloves / protective clothing / eye protection. P403+P235 Store in a well-ventilated place. Keep cool.
Response		P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]. P370+P378 In case of fire: Use CO2, powder or water spray to extinguish.
Disposal		P501 This material and its container must be disposed of in a safe way.

Product Identifier: FHRW130	**Trade Name**	REAG 2
	Hazardous Substance	SODIUM AZIDE (EINECS: 247-852-1, CAS: 26628-22-8)
Classification		Not Classified
Hazard Pictogram		None
Signal Word		None
Hazard Statement		EUH032: Contact with acids liberates very toxic gas.
Precautionary Statements		
Prevention		None
Response		None
Disposal		None

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 Liquid Stable (LS) 2-Part Homocysteine Reagent Assay.
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.

RESULTS

Results are reported in µmol/L. Specimens >44 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. Ensure results are multiplied by the correct dilution factor.

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 Liquid Stable (LS) 2-Part Homocysteine Reagent Assay is 2-44 µmol/L.

LIMITATIONS OF USE

1. In Vitro Diagnostic Use. For professional use only.
2. The linear range of the Liquid Stable (LS) 2-Part Homocysteine Reagent Assay when run as directed is 2-44 µmol/L for the AU Platforms. Specimens > 44 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.
3. The Reagents should be clear. Discard if turbid.
4. 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]
5. Carbamazepine, methotrexate, phenytoin, nitrous oxide, or 6-azauridine triacetate may affect the homocysteine concentration.[16]
6. 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.
7. 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.
8. Limitations: Hydroxlamine, present in several iron reagents may carryover (via reagent probe/mixers or reaction cuvette) and cause false low results. Routine rinsing procedures are not adequate to eliminate this problem in most cases (including the Beckman Coulters UIBC reagent (P/N OSR1205, which contains Hydroxylamine). Please refer to Axis Shield Contamination Avoidance protocol for the prevention of carryover on AU systems.
   Please ensure that the appropriate contamination avoidance parameters have been implemented. Analyzer specific contamination avoidance parameters are available from Axis-Shield Customer Support.
9. Ethanol vapour may be released from the Homocysteine REAG 1 Reagent when on-board the reagent carousel of BECKMAN COULTER AU series analysers. Avoid the use of ethanol reagents together with Homocysteine to avoid potential contamination through atmospheric means.
10. Not tested for use on paediatric patients

PERFORMANCE DATA

BASED ON MEASUREMENTS GENERATED ON BECKMAN COULTER AU PLATFORMS - AU400, AU480, AU680, AU5800, DxC 500 AU AND DxC 700 AU

Accuracy

A correlation study was performed with plasma specimens from apparently healthy adults. All specimens were analysed using the Liquid Stable (LS) 2-Part Homocysteine Reagent according to the CLSI (formally NCCLS) document EP9-A2[27] or CLSI document EP9-A3[31]. All results are described using a 95% confidence Interval. Specimen ranges and data gave:

Comparison Method	Beckman Coulter AU400 ***vs.Catch Liquid Stable***	Beckman Coulter AU480 ***vs AU400***	Beckman Coulter AU680 ***vs AU400***	Beckman Coulter AU5800 ***vs AU400***	Beckman Coulter DxC 500 AU vs **AU480**	Beckman Coulter DxC 700 AU vs **AU400**
CLSI Document used	EP9-A2	EP9-A2	EP9-A2	EP9-A2	EP9-A3	EP9-A2
Number of specimens	94	99	98	99	105	94
Slope of regression line	0.99	0.97	0.97	0.98	0.98	0.99
Y-Intercept	0.17	-0.68	-0.22	-0.75	0.40	0.67
Correlation coefficient	1.00	1.00	1.00	1.00	0.98	1.00
Sample Range	6.5 – 49.0	8.5 – 45.1	8.5 – 45.1	8.5 – 45.1	3.1 – 41.3	5.8 – 45.9

Precision

Studies on the AU Platforms (AU400, AU480, AU680, AU5800, DxC 500 AU and DxC 700 AU) were performed with guidance from the CLSI (formally NCCLS) Document EP5-A2[28] or CLSI document EP5-A3[32]. 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 on a minimum of 5 days. Results are summarised below:

Beckman Coulter AU400

Sample	n	Reagent Lot	Mean	Within-Run		Between-Run		Total	
				SD	%CV	SD	%CV	SD	%CV
Low Control	80	1	6.28	0.17	2.6	0.11	1.7	0.28	4.4
	80	2	6.29	0.13	2.1	0.11	1.7	0.26	4.1
Medium Control	80	1	12.33	0.18	1.5	0.15	1.2	0.37	3.0
	80	2	12.24	0.16	1.3	0.16	1.3	0.39	3.2
High Control	80	1	25.53	0.38	1.5	0.35	1.4	0.65	2.5
	80	2	25.27	0.41	1.6	0.00	0.0	0.73	2.9
Sample P1	80	1	6.67	0.13	1.9	0.00	0.0	0.23	3.3
	80	2	6.97	0.15	2.2	0.00	0.0	0.31	4.4
Sample P2	80	1	35.96	0.46	1.3	0.40	1.1	0.89	2.5
	80	2	35.53	0.40	1.1	0.23	0.7	0.82	2.3
Sample P3	80	1	48.31	0.53	1.1	0.42	0.9	0.97	2.0
	80	2	47.66	0.47	1.0	0.38	0.8	1.07	2.2

Beckman Coulter AU480

Sample	n	Reagent Lot	Mean	Within-Run		Between-Run		Total	
				SD	%CV	SD	%CV	SD	%CV
Low Control	20	1	6.73	0.07	1.1	0.17	2.6	0.21	3.1
	20	2	6.51	0.17	2.5	0.11	1.7	0.22	3.4
Medium Control	20	1	12.74	0.18	1.4	0.13	1.0	0.24	1.9
	20	2	12.43	0.22	1.8	0.17	1.3	0.30	2.4
High Control	20	1	26.13	0.24	0.9	0.11	0.4	0.46	1.8
	20	2	25.66	0.17	0.7	0.12	0.5	0.47	1.8
Sample P1	20	1	10.54	0.33	3.1	0.00	0.0	0.37	3.5
	20	2	11.00	0.71	6.5	0.00	0.0	0.92	8.4
Sample P2	20	1	28.71	0.24	0.9	0.18	0.6	0.58	2.0
	20	2	28.20	0.18	0.6	0.12	0.4	0.60	2.1
Sample P3	20	1	37.63	0.32	0.9	0.18	0.5	0.97	2.6
	20	2	36.98	0.21	0.6	0.12	0.5	0.91	2.5

Beckman Coulter AU680

Sample	n	Reagent Lot	Mean	Within-Run		Between-Run		Total	
				SD	%CV	SD	%CV	SD	%CV
Low Control	20	1	6.96	0.16	2.4	0.00	0.0	0.16	2.4
	20	2	6.79	0.16	2.3	0.02	0.3	0.21	3.1
Medium Control	20	1	13.03	0.12	1.0	0.15	1.2	0.20	1.5
	20	2	12.76	0.20	1.6	0.05	0.4	0.22	1.7
High Control	20	1	26.38	0.23	0.9	0.28	1.0	0.41	1.6
	20	2	26.19	0.31	1.2	0.24	0.9	0.40	1.5
Sample P1	20	1	10.76	0.30	2.8	0.00	0.0	0.32	3.0
	20	2	10.65	0.32	3.0	0.00	0.0	0.39	3.6
Sample P2	20	1	28.90	0.34	1.2	0.15	0.5	0.48	1.6
	20	2	28.67	0.42	1.5	0.06	0.5	0.73	2.5
Sample P3	20	1	37.78	0.28	0.7	0.16	0.4	0.51	1.4
	20	2	37.90	0.28	0.7	0.11	0.3	0.67	1.8

Beckman Coulter AU5800

Sample	n	Reagent Lot	Mean	Within-Run		Between-Run		Total	
				SD	%CV	SD	%CV	SD	%CV
Low Control	20	1	6.49	0.24	3.6	0.00	0.0	0.30	4.7
	20	2	6.70	0.13	2.2	0.07	1.1	0.16	2.7
Medium Control	20	1	12.52	0.23	1.8	0.00	0.0	0.23	1.8
	20	2	12.57	0.17	1.4	0.19	1.5	0.26	2.1
High Control	20	1	25.87	0.26	1.0	0.32	1.2	0.41	1.6
	20	2	25.69	0.30	1.2	0.16	0.6	0.34	1.3
Sample P1	20	1	10.53	0.16	1.5	0.00	0.0	0.35	3.3
	20	2	10.53	0.27	2.6	0.00	0.0	0.34	3.2
Sample P2	20	1	28.58	0.22	0.8	0.24	0.8	0.52	1.8
	20	2	28.42	0.29	1.0	0.07	0.3	0.49	1.7
Sample P3	20	1	37.67	0.35	0.9	0.27	0.7	0.79	2.1
	20	2	37.55	0.29	0.8	0.26	0.7	0.55	1.5

Beckman Coulter DxC 500 AU

Sample	n	Reagent Lot	Mean	Within-Run		Between-Run		Total	
				SD	%CV	SD	%CV	SD	%CV
Low Control	80	1	5.83	0.14	2.3%	0.29	5.0%	0.29	4.9%
	80	2	6.46	0.15	2.3%	0.38	5.9%	0.38	5.8%
Medium Control	80	1	11.60	0.14	1.2%	0.54	4.7%	0.53	4.6%
	80	2	11.92	0.21	1.7%	0.51	4.2%	0.48	4.1%
High Control	80	1	23.59	0.24	1.0%	0.63	2.7%	0.62	2.6%
	80	2	24.24	0.24	1.0%	0.75	3.1%	0.74	3.0%
Sample P1	80	1	9.63	0.36	3.7%	0.49	5.1%	0.44	4.5%
	80	2	9.39	0.18	2.0%	0.46	4.9%	0.45	4.8%
Sample P2	80	1	30.01	0.63	2.1%	1.01	3.3%	0.94	3.1%
	80	2	28.09	0.28	1.0%	0.87	3.1%	0.86	3.1%
Sample P3	80	1	40.53	1.14	2.8%	1.61	4.0%	1.44	3.6%
	80	2	37.18	0.33	0.9%	1.13	3.0%	1.11	3.0%

Beckman Coulter DxC 700 AU

Sample	n	Reagent Lot	Mean	Within-Run		Between-Run		Total	
				SD	%CV	SD	%CV	SD	%CV
Low Control	80	1	6.96	0.1	1.7	0.0	0.0	0.3	5.1
	80	2	6.79	0.1	2.1	0.1	1.6	0.3	4.8
Medium Control	80	1	13.03	0.1	1.1	0.0	0.0	0.4	3.0
	80	2	12.76	0.2	1.4	0.0	0.0	0.4	3.6
High Control	80	1	26.38	0.2	0.9	0.0	0.0	0.6	2.4
	80	2	26.19	0.2	0.8	0.1	0.5	0.6	2.7
Sample P1	80	1	10.76	0.2	2.2	0.2	1.7	0.4	3.9
	80	2	10.65	0.2	2.2	0.2	2.2	0.4	4.1
Sample P2	80	1	28.90	0.5	1.5	0.2	0.6	0.7	2.5
	80	2	28.67	0.5	1.6	0.3	1.1	0.8	2.8
Sample P3	80	1	37.78	0.5	1.2	0.2	0.6	0.9	2.2
	80	2	37.90	0.6	1.5	0.0	0.0	1.0	2.6

Dilution Linearity

The dilution linearity of the Liquid Stable (LS) 2-Part Homocysteine Reagent Assay on the Beckman AU platforms gives a % recovery of 100 ± 10% for all samples across the range of the assay. Samples >44 µmol/L exhibit mean recovery of 100% ± 11% of all the expected results when diluted into the assay range.

Limit of Detection

The limit of detection (LOD) of each system was determined according to CLSI (formally NCCLS) Document EP17-A[29] or EP17-A2[33]* *LOD values (µmol/L) are tabulated below:*

Beckman Coulter AU400	*Beckman Coulter AU480*	*Beckman Coulter AU680*	*Beckman Coulter AU5800*	*Beckman Coulter DxC 500 AU**	*Beckman Coulter DxC 700 AU*
0.33	*0.39*	*0.54*	*0.59*	*0.89*	*1.04*

**CLSI Document EP17-A2*

Analytical Specificity

Analytical specificity was assessed only on the Beckman Coulter AU400 based on the 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
Red Blood Cells	0.4%	≤ +10
Triglyceride	500 mg/dL	≤ +10
Glutathione	1000 µmol/L	≤ +10
Methionine	800 µmol/L	≤ +10
L-Cysteine	200 µmol/L	≤ +10
Pyruvate	1250 µmol/L	≤ +10

None of these substances interfered significantly in the assay.

Samples with raised protein levels show >10% difference compared to results obtained from normal samples and should be avoid.
Refer to Reference 16 in the references section of this pack leaflet for possible interferences caused by drugs, disease or preanalytical variables.

Sample Carryover

Sample carryover studies on all AU Platforms tested show that carryover is less than the limit of detection of the assay.

On-board Reagent Stability

The reagents are stable for 30 days on all AU Platforms.

Calibration Stability

The calibration curve is stable for up to 30 days as verified on the Beckman Coulter AU400 and up to 14 days as verified on the Beckman Coulter AU5800, DxC 500 AU & DxC 700 AU.

Specimen Types

The specimen collection tubes verified to be used are EDTA and lithium heparin plasma tubes, serum and Serum Separator tubes. Other specimen collection tubes have not been tested.

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. It is the responsibility of the operator to verify that the correct tubes are used. 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]

AU PLATFORM ASSAY PROTOCOLS – AU400, AU480, AU680, AU5800, DxC 500 AU and DxC 700 AU

Ensure that the assay parameters exactly match those listed below.

AU400 – PROCEDURE PARAMETERS

Test No. [*]	Name [HCY]	Type [Ser.]	
Sample Volume:	[16.5] µL	Diluent Volume:	[0.0] µL
Pre-Dilution Factor:	[1]		
Reagent 1 Volume:	[250] µL	Diluent Volume:	[0.0] µL
Reagent 2 Volume:	[25] µL	Diluent Volume:	[0.0] µL
Wavelength Pri:	[340] nm		
Wavelength Sec:	[380] nm		
Reaction Method:	RATE1		
Reaction Slope	[-]		
Point 1	Fst [15]		
	Lst [27]		
Point 2	Fst [ ]		
	Lst [ ]		
Linearity	[100]%		
No-Lag-Time	[No]		
Min. OD		Max. OD	
L [-2.0]		H [2.5]	
Reagent OD Limit	Fst L [ ]	Fst H [ ]	
	Lst L [ ]	Lst H [ ]	
Dynamic Range:	L [2.0]	H [44.0]	
Correlation Factor:	A [1.0]	B [0.0]	
Onboard Stability Period:		[30]	
Calibration Specific:			
	Point	OD	Conc
	1 [*]	[ ]	[0.0]
	2 [*]	[ ]	[**]
	Calibration Type:		[AA]
	Formula:	[Y=AX+B]	

*User Defined **Enter Values on Calibrator Vials

AU480 / AU680– PROCEDURE PARAMETERS

Test No. [*]	Name [HCY]	Type [Ser.]	
Sample Volume:	[10] µL	Diluent Volume:	[0.0] µL
Pre-Dilution Factor:	[1]		
Reagent 1 Volume:	[155] µL	Diluent Volume:	[0.0] µL
Reagent 2 Volume:	[16] µL	Diluent Volume:	[0.0] µL
Wavelength Pri:	[340] nm		
Wavelength Sec:	[380] nm		
Reaction Method:	RATE1		
Reaction Slope	[-]		
Point 1	Fst [15]		
	Lst [27]		
Point 2	Fst [ ]		
	Lst [ ]		
Linearity	[25]%		
No-Lag-Time	[Yes]		
Min. OD		Max. OD	
L […]		H […]	
Reagent OD Limit	Fst L [-2.0]	Fst H [2.5]	
	Lst L [-2.0]	Lst H [2.5]	
Dynamic Range:	L [2.0]	H [44.0]	
Correlation Factor:	A [1.0]	B [0.0]	
Onboard Stability Period:		[30]	
LIH Influence Check		[No]	
Calibration Specific:			
	Point	OD	Conc
	1 [*]	[ ]	[0.0]
	2 [*]	[ ]	[**]
	Calibration Type:		[AA]
	Formula:	[Y=AX+B]	
Stability	Reagent Blank [30] day	Calibration [14] Day	

*User Defined **Enter Values on Calibrator Vials

AU5800– PROCEDURE PARAMETERS

Test No. [*]	Name [HCY]	Type [Ser.]	
Sample Volume:	[7.5] µL	Diluent Volume:	[0.0] µL
Pre-Dilution Factor:	[1]		
Reagent 1 Volume:	[115] µL	Diluent Volume:	[0.0] µL
Reagent 2 Volume:	[12] µL	Diluent Volume:	[0.0] µL
Wavelength Pri:	[340] nm		
Wavelength Sec:	[380] nm		
Reaction Method:	RATE1		
Reaction Slope	[-]		
Point 1	Fst [15]		
	Lst [27]		
Point 2	Fst [ ]		
	Lst [ ]		
Linearity	[25]%		
No-Lag-Time	[Yes]		
Min. OD		Max. OD	
L [ ]		H [ ]	
Reagent OD Limit	Fst L [-2.0]	Fst H [2.5]	
	Lst L [-2.0]	Lst H [2.5]	
Dynamic Range:	L [2.0]	H [44.0]	
Correlation Factor:	A [1.0]	B [0.0]	
Onboard Stability Period:		[30]	
LIH Influence Check		[No]	
Calibration Specific:			
	Point	OD	Conc
	1 [*]	[ ]	[0.0]
	2 [*]	[ ]	[**]
	Calibration Type:		[AA]
	Formula:	[Y=AX+B]	
Stability	Reagent Blank [30] day	Calibration [14] Day	

*User Defined **Enter Values on Calibrator Vials

DxC 500 AU- PROCEDURE PARAMETERS

Test No. [*]	Name [HCY]	Type [Ser.]	
Sample Volume:	[10] µL	Diluent Volume:	[0.0] µL
Pre-Dilution Factor:	[1]		
Reagent 1 Volume:	[155] µL	Diluent Volume:	[0.0] µL
Reagent 2 Volume:	[16] µL	Diluent Volume:	[0.0] µL
Wavelength Pri:	[340] nm		
Wavelength Sec:	[380] nm		
Reaction Method:	RATE1		
Reaction Slope	[-]		
Point 1	Fst [15]		
	Lst [27]		
Point 2	Fst [ ]		
	Lst [ ]		
Linearity	[25]%		
No-Lag-Time	[Yes]		
Min. OD		Max. OD	
L [-2.0]		H [2.5]	
Reagent OD Limit	Fst L [-2.0]	Fst H [2.5]	
	Lst L [-2.0]	Lst H [2.5]	
Dynamic Range:	L [2.0]	H [44.0]	
Correlation Factor:	A [1.0]	B [0.0]	
Onboard Stability Period:		[30]	
LIH Influence Check		[No]	
Calibration Specific:			
	Point	OD	Conc
	1 [*]	[ ]	[0.0]
	2 [*]	[ ]	[28]
	Calibration Type:		[AA]
	Formula:	[Y=AX+B]	
Stability	Reagent Blank [30] day	Calibration [14] Day	

Values set for working in µmol *User Defined

DxC 700 AU-ASSAY PROCEDURE PARAMETERS

Test Name.		Name [HCY1G]	Reagent ID [225]	
Sample Volume:		[10] µL	Diluent	[0.0] µL
Pre-Dilution Factor:		[1]		
Reagent 1 Volume (R1):		[155] µL	Diluent	[0.0] µL
Reagent 2 Volume (R2):		[16] µL	Diluent	[0.0] µL
Wavelength Pri:		[340] nm		
Wavelength Sec:		[380] nm		
Reaction Method:		RATE1		
Reaction Slope		[-]		
Measuring Point-1		1st [15]	Last [27]	
Measuring Point-2		1st [ ]	Last [ ]	
Linearity		[25]%		
Lag Time Check		[Yes]		
Min. OD		[-2.0]	Max. OD	[3.0]
Reagent OD Limit		1st C [-2.0]	C [2.5]	
		Last L [-2.0]	C [2.5]	
Analytical Measuring Range		C* [2.0]	C* [44.0]	
Correlation Factor:		A [1]	B [0]	
Onboard Stability Period:			[30]	
LIH Influence Check:			[No]	
Value/Flag		[Value]		
Low		[-9999999]	High	[9999999]
Critical Limits		Low [-9999999]	High [9999999]	Unit [µmol/L]
Decimal Places		[1]		
Test Name:		HCY1G]	HCY1G]	[Serum]
Calibration Type		[AA]	Formula	[Y=AX+B]
Counts		[2]		
Point-1	[Cal0]	Conc [0]	Low [9999999]	High [9999999]
Point-1	[Cal28]	Conc [28]	Low [9999999]	High [9999999]
Slope Check	[None]	Advanced Calibration Operation		[No]
Stability Reagent Blank		[30] Day	[0] Hour	

* Values set for working in µmol

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The Technology Park
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United Kingdom
Tel: +44 (0) 1382 422000
Fax: +44 (0) 1382 422088

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EC Importer for Beckman Coulter:
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