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). 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).

Hyperhomocysteinemia, elevated levels of homocysteine, can be associated with an increased risk of cardiovascular disease (CVD). 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.

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.

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 NADH as coenzyme. The rate of NADH conversion to NAD+ (\( \Delta A_{340\,nm} \)) is directly proportional to the concentration of homocysteine (D\( A_{340\,nm} \)).

**SUMMARY AND EXPLANATION OF TEST**

**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 NADH as coenzyme. The rate of NADH conversion to NAD+ (\( \Delta A_{340\,nm} \)) is directly proportional to the concentration of homocysteine (D\( A_{340\,nm} \)).

**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).

**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.

**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, 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.

**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.

**REFERENCES**

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:

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

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. Additionally matrix differences between serum and serum separator tubes and plasma tubes have been reported. 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.
   - All specimens may be kept on ice for up to 6 hours prior to separation by centrifugation.
   - 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.

   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.

   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.

   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. HCY values will normally increase with age, giving a reference range among an elderly population (> 60 years) of 5-20 µmol/L. In countries with folic acid fortification programmes, reduced levels of HCY may be observed.

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.

4. Carbamazepine, methotrexate, phenytoin, nitrous oxide, or 6-azauridine triacetate may affect the homocysteine concentration.

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 (formerly NCCLS) document EP9-A2. 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:

<table>
<thead>
<tr>
<th>Comparison Method</th>
<th>Beckman Coulter AU 400 v. SYNCHRON UniCel DxC 600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of specimens</td>
<td>50</td>
</tr>
<tr>
<td>Slope of regression line</td>
<td>0.99</td>
</tr>
<tr>
<td>Y-Intercept</td>
<td>0.74</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.994</td>
</tr>
</tbody>
</table>

3
**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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reagent Lot</th>
<th>Mean</th>
<th>Within-Run</th>
<th>Between-Run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>%CV</td>
<td>SD</td>
<td>%CV</td>
</tr>
<tr>
<td>Low Control</td>
<td>1</td>
<td>6.15</td>
<td>0.36</td>
<td>5.9</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.57</td>
<td>0.30</td>
<td>4.7</td>
<td>0.00</td>
</tr>
<tr>
<td>Medium Control</td>
<td>1</td>
<td>11.65</td>
<td>0.49</td>
<td>4.2</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.90</td>
<td>0.33</td>
<td>2.8</td>
<td>0.21</td>
</tr>
<tr>
<td>High Control</td>
<td>1</td>
<td>24.13</td>
<td>0.64</td>
<td>2.6</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>24.37</td>
<td>0.63</td>
<td>2.6</td>
<td>0.59</td>
</tr>
<tr>
<td>Sample P1</td>
<td>1</td>
<td>7.43</td>
<td>0.47</td>
<td>6.3</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.63</td>
<td>0.27</td>
<td>3.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Sample P2</td>
<td>1</td>
<td>33.20</td>
<td>0.85</td>
<td>2.6</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>33.58</td>
<td>0.79</td>
<td>2.4</td>
<td>0.65</td>
</tr>
<tr>
<td>Sample P3</td>
<td>1</td>
<td>45.38</td>
<td>1.08</td>
<td>2.4</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>45.61</td>
<td>0.96</td>
<td>2.1</td>
<td>0.62</td>
</tr>
</tbody>
</table>

---

**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-A29 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 EP17-A29 for the interfering substances listed in the table below:

<table>
<thead>
<tr>
<th>Interfering Substance</th>
<th>Concentration</th>
<th>% Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>20 mg/dL</td>
<td>≤ ±10</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>500 mg/dL</td>
<td>≤ ±10</td>
</tr>
<tr>
<td>Tri glyceride</td>
<td>1000 mg/dL</td>
<td>≤ ±10</td>
</tr>
<tr>
<td>Glutathione</td>
<td>1000 µmol/L</td>
<td>≤ ±10</td>
</tr>
<tr>
<td>Methionine</td>
<td>800 µmol/L</td>
<td>≤ ±10</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>200 µmol/L</td>
<td>≤ ±10</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>1250 µmol/L</td>
<td>≤ ±10</td>
</tr>
<tr>
<td>Total Protein</td>
<td>120 mg/mL</td>
<td>≤ ±10</td>
</tr>
</tbody>
</table>

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. Additionally matrix differences between serum, Serum Separator tubes and plasma tubes have been reported.

---

[16] Reference 16

---


---


---


---


---


---

Assay Name: HCTX

### Chemistry Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Type:</td>
<td>Rate 1</td>
</tr>
<tr>
<td>Units:</td>
<td>µmol/L</td>
</tr>
<tr>
<td>Precision:</td>
<td>X.XX</td>
</tr>
<tr>
<td>Reaction Direction:</td>
<td>Negative</td>
</tr>
<tr>
<td>Math Model:</td>
<td>Linear</td>
</tr>
<tr>
<td>Primary Wavelength:</td>
<td>340</td>
</tr>
<tr>
<td>Secondary Wavelength:</td>
<td>380</td>
</tr>
<tr>
<td>Calculation Factor:</td>
<td>1.000</td>
</tr>
<tr>
<td>No. of Calibrators:</td>
<td>2</td>
</tr>
<tr>
<td>Setpoints:</td>
<td>1 0.000</td>
</tr>
<tr>
<td>Cal Time Limit:</td>
<td>336 hours</td>
</tr>
</tbody>
</table>

### Processing Parameters

<table>
<thead>
<tr>
<th>Component</th>
<th>First Inject</th>
<th>Second Inject</th>
<th>Third Inject</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Component</td>
<td>Calculate Time</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>185 µL</td>
<td>-180 sec</td>
<td>550 sec</td>
</tr>
<tr>
<td>B</td>
<td>70 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>38 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dispense Volume</td>
<td>Blank</td>
<td>Reaction 1</td>
<td>Reaction 2</td>
</tr>
<tr>
<td>Start Read</td>
<td>-50 sec</td>
<td>-10 sec</td>
<td></td>
</tr>
<tr>
<td>End Read</td>
<td>600 sec</td>
<td>720 sec</td>
<td></td>
</tr>
<tr>
<td>Usable Result Range</td>
<td>1.000</td>
<td>50.000</td>
<td></td>
</tr>
</tbody>
</table>

### Error Detection Limits

<table>
<thead>
<tr>
<th>Limit Type</th>
<th>Blank</th>
<th>Reaction 1</th>
<th>Reaction 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABS Low Limit</td>
<td>-1.500</td>
<td>-1.500</td>
<td>-1.500</td>
</tr>
<tr>
<td>ABS High Limit</td>
<td>2.200</td>
<td>2.200</td>
<td>2.200</td>
</tr>
<tr>
<td>Rate Low Limit</td>
<td>2.200</td>
<td>2.200</td>
<td>-1.500</td>
</tr>
<tr>
<td>Rate High Limit</td>
<td>-1.500</td>
<td>-1.500</td>
<td>2.200</td>
</tr>
<tr>
<td>Mean Deviation</td>
<td>2.200</td>
<td>2.200</td>
<td>2.200</td>
</tr>
</tbody>
</table>

Beckman Coulter, SYNCHRON and UniCel are trademarks of Beckman Coulter, Inc. and are registered in the USPTO. All other trademarks are the property of their respective owners.
REFERENCES


In Vitro Diagnostic Medical Device 
Store at 2-8°C

Product code
Manufactured by

Lot number
Store in the dark

100 tests
Reagent 1, 2, 3

Consult Instructions For Use
Calibrator 0 µmol/L, Calibrator 28 µmol/L

Use by

Rx Only
Prescription Use Only

Ver: 2016/05
RPBL1054/R5