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.

PRINCIPLE OF THE ASSAY
This assay consists of two key steps:

Reduction: Dimerised homocysteine, mixed disulphide, and protein-bound forms of Homocysteine (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 (CBS) and excess serine. The cystathionine is then broken down to homocysteine, pyruvate and ammonia via cystathionine beta reductase (1) and reductase II is converted to lactate via lactate dehydrogenase (LDH) with nicotinamide adenine dinucleotide (NADH) as coenzyme. The rate of NADH conversion to NAD+ (measured at A340 nm) is directly proportional to the concentration of homocysteine.

KIT COMPONENTS

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REAG 1</td>
<td>1 x 30.0 mL</td>
<td>NADH (0.47 mM), LDH (38 KU/L), Trizma Base 1-10%, Trizma Hydrochloride 1-10%, Reductant (TCEP:2.9 mM)</td>
</tr>
<tr>
<td>REAG 2</td>
<td>1 x 5.0 mL</td>
<td>Cycling Enzymes; CBS (0.748 KUL) and CBL (16.4 KUL)</td>
</tr>
<tr>
<td>CAL (Blue Cap)</td>
<td>1 x 3.0 mL</td>
<td>Homocysteine blank (0 µmol/L), Ready-to-use</td>
</tr>
<tr>
<td>CAL (Red Cap)</td>
<td>1 x 3.0 mL</td>
<td>Homocysteine solution (28 µmol/L), Ready-to-use</td>
</tr>
</tbody>
</table>

STANDARDISATION
The calibrators are traceable to NIST SRM 1955, confirmed by a designated measurement procedure (HPLC).

ITEMS REQUIRED BUT NOT PROVIDED
An analyzer capable of dispensing 2 reagents and measuring absorbance at 340 nm with temperature control (37°C).

An Axis-Shield Homocysteine Control Kit (FHCY200) is sold separately and is available for use with the Liquid Stable (LS) 2-Part Homocysteine Reagent.

STORAGE OF REAGENTS, HANDLING AND PROCEDURAL NOTES
1. Store kit components at 2-8°C and use until the expiry date on the labels.
2. Do not use expired reagents.
3. Do not mix different reagent kit lots.
4. Do not expose reagents to light during on-board use.
5. Avoid contamination of reagents. Use a new disposable pipette tip for each reagent or sample manipulation.
6. Reagents should be clear of particulate matter and should be discarded if they become turbid.

WARNINGS AND SAFETY PRECAUTIONS
1. 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.
2. Material safety data sheets are available upon request from Axis-Shield.

ASSAY PROCEDURE
- Programme instrument using appropriate instrument protocols.
- Load reagents and samples onto the instrument as instructed.
- Run assay.

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.
2. 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.
3. 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.
3. Specimens should be stored at 2-8°C for 8 months. Mix specimens thoroughly after thawing. Avoid multiple freeze-thawing.
4. 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.

QUALITY CONTROL PROCEDURES
Maintenance and calibration of the instrument must be performed according to the manufacturer’s instructions. Assayed control materials with values for homocysteine in both the normal and abnormal ranges should be tested to validate reagent performance. Users should demonstrate that they obtain performance specifications for precision and reportable range of test results comparable to those established by the manufacturer before reporting patient test results.

EXPECTED VALUES
The reference range should be determined by each laboratory. HCY concentrations in healthy individuals varies with age, gender, geographical areas and genetic factors. Scientific literature reports reference values for adult male and females between 5-15 µmol/L. A reference range among an elderly population (> 60 years) is 8-20 µmol/L in countries with folate fortification programmes, reduced levels of HCY may be observed. As a point of reference the ranges quoted above may be used until the laboratory has analysed a sufficient number of specimens to determine its own reference range.

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 when run as directed is 1-46 µmol/L for the OLYMPUS AU400 and COBAS Integra 800 and 2-46 µmol/L for the ROCHE Hitachi 917 and ROCHE Modular P.
3. Specimens >46 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.
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.
5. Hydroxyalnine, present in several iron reagents may carryover (reagent probe or reaction cuvette) and cause falsely low results. Routine rinsing procedures are not adequate to eliminate this problem in most cases. Possible solutions would include special washing protocols, changing to an iron assay that used ascorbic acid as reductant or running iron and homocysteine assays on separate instruments.
6. Carbamazepine, methotrexate, phenytoin, nitrous oxide, or 6-azauridine triacetate may affect the homocysteine concentration.
7. Note: Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine.
8. 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.

RESULTS
The results are calculated automatically and are presented in µmol/L. Ensure results are multiplied by the correct dilution factor.
PERFORMANCE DATA

Data presented were generated on the OLYMPUS AU400, COBAS INTEGRA 800, ROCHE Hitachi 917 and ROCHE Modular P systems. Results may vary depending on the system used. Other instrument protocols are available. It is the responsibility of the user to verify performance. See www.homocysteine.org.uk or contact the manufacturer.

Accuracy:

A correlation study was performed to a comparator device based on guidance from NCCLS document EP9-A2.1. The specimens tested gave the statistical values (95% confidence intervals) as summarized below:

<table>
<thead>
<tr>
<th>Instrument System</th>
<th>Specimen Range (µmol/L)</th>
<th>Specimen CV (%)</th>
<th>Mean Recovery (%)</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLYMPUS AU400</td>
<td>6.5–49.0</td>
<td>94</td>
<td>0.99</td>
<td>0.17</td>
</tr>
<tr>
<td>COBAS Integra 800</td>
<td>6.3–48.4</td>
<td>100</td>
<td>0.97</td>
<td>-0.16</td>
</tr>
<tr>
<td>ROCHE Hitachi 917</td>
<td>8.2–45.6</td>
<td>96</td>
<td>0.97</td>
<td>0.49</td>
</tr>
<tr>
<td>ROCHE Modular P</td>
<td>5.7–47.1</td>
<td>96</td>
<td>0.94</td>
<td>-0.22</td>
</tr>
</tbody>
</table>

Precision:

A 20-day study was performed based on guidance from NCCLS document EP9-A2.1 using two reagent lots and a stored calibration curve. Results (rounded to 1 decimal place) per system are summarized below for each level tested (n=80).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean µmol/L</th>
<th>Within Run CV (%)</th>
<th>Total CV (%)</th>
<th>Mean µmol/L</th>
<th>Within Run CV (%)</th>
<th>Total CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel 1</td>
<td>7.0</td>
<td>2.2</td>
<td>4.4</td>
<td>8.5</td>
<td>1.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Panel 2</td>
<td>36.0</td>
<td>1.3</td>
<td>2.3</td>
<td>35.9</td>
<td>0.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Panel 3</td>
<td>48.8</td>
<td>1.1</td>
<td>2.0</td>
<td>45.6</td>
<td>0.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Low Control</td>
<td>4.7</td>
<td>1.0</td>
<td>2.2</td>
<td>4.5</td>
<td>0.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Medium Control</td>
<td>6.9</td>
<td>2.3</td>
<td>4.4</td>
<td>6.0</td>
<td>2.6</td>
<td>3.0</td>
</tr>
<tr>
<td>High Control</td>
<td>25.5</td>
<td>1.5</td>
<td>2.5</td>
<td>23.4</td>
<td>1.1</td>
<td>1.9</td>
</tr>
<tr>
<td>ROCHE Hitachi 917</td>
<td>29.5</td>
<td>1.8</td>
<td>2.9</td>
<td>23.4</td>
<td>1.9</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Dilution Linearity

Test No. | Name [HCV] | Type [Ser.] | Sample Volume | Diluent Volume |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>[16.5] µL</td>
<td>[0.0] µL</td>
</tr>
<tr>
<td>Pre-Dilution Factor:</td>
<td>[1]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent 1 Volume:</td>
<td>[250] µL</td>
<td>[0.0] µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent 2 Volume:</td>
<td>[25] µL</td>
<td>[0.0] µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wavelength:</td>
<td>[385] nm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction Method:</td>
<td>RATE1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction Slope:</td>
<td>[1]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Point 1</td>
<td>[15]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Point 2</td>
<td>[27]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Point 3</td>
<td>[19]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linearly</td>
<td>[1.0]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-Lag Time:</td>
<td>[No]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. OD:</td>
<td>[3.2]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. OD:</td>
<td>[10]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L: [2.0]</td>
<td>H: [2.5]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent OD Limit:</td>
<td>[Ft]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation Factor:</td>
<td>A [1.0]</td>
<td>B [0.0]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibration Specific:</td>
<td>[30]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula:</td>
<td>[Y=AX+B]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ASSAY PROTOCOLS

ENSURE THAT THE USER DEFINED* ASSAY PROCEDURE PARAMETERS ENTER MATCH EXACTLY THOSE LISTED FOR THE SYSTEM

OLYMPUS AU400 – PROCEDURE PARAMETERS

Analytical Specificity:

The specificity was assessed on the Olympus AU400 based on guidance from CLSI EP7-A2.1 for the interfering substances tabulated below:

<table>
<thead>
<tr>
<th>Interfering Substance</th>
<th>Interfering Substance Concentration</th>
<th>% Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>≤ 20 mg/dL</td>
<td>≤ 10</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>500 µg/dL</td>
<td>≤ 10</td>
</tr>
<tr>
<td>Triglyceride (lauric acid)</td>
<td>500 mg/dL</td>
<td>≤ 10</td>
</tr>
<tr>
<td>Creatinine</td>
<td>500 µmol/L</td>
<td>≤ 10</td>
</tr>
<tr>
<td>Cysteine</td>
<td>200 µmol/L</td>
<td>≤ 20</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>1250 µmol/L</td>
<td>≤ 20</td>
</tr>
</tbody>
</table>

Samples with raised protein levels show >10% difference compared to results obtained from normal samples and should be avoided. None of these substances interfered significantly in the assay.

LITERATURE REFERENCES


User Defined

User Defined: Enter Values on Calibrator Vials

[1] EDTA samples can be stored on board the instrument for 3 hours, other have not been tested.
ROCHE HITACHI 917 – PROCEDURE PARAMETERS

Test: HCY*  
Type: Ser/Pl

ANALYZE

Assay time/Point  [2 Point End][10][19][34][0][0]
Wave (2nd/Primary)  [376][340]
S. Vol (Normal)  [16.5]
Reagent (R1) T1  [250][0] [000000]
Reagent (R2) T2  [0][0] [000000]
Reagent (R3) T3  [25][0] [000000]
Abs. Limit  [32000] [Decrease] 2 Tests
Prozone Limit  [32000] [0] [Lower]
Cell Detergent (Detergent 1)
CALIB
Calibration Type  [Linear]
Point  [2]
Span Point  [2]
Weight  [0]
Auto Calibration  
2 Point  [720]
SD Limit  [100]
Duplicate Limit  [10%] [32000 Abs]
Sensitivity Limit  [-99999] [99999]
S1 Abs limit  [32000] [32000]
RANGE
Application Code*  [ ] Unit [µmol/L]
Control Interval*  [ ]
Instrument Factor  [Y=aX+b] a=1.0 b=0.0
Technical Limit  [2.0] [46.0]
Repeat Limit*  [-99999] [99999]
OTHERS
<Standard>  (1) (2)
Calibration Code*  [ ] [ ]
Concentration**  [0.00] [**]
Position*  [ ] [1]
Sample Volume  [16.5] [16.5]

ROCHE MODULAR ANALYTICS -P> – PROCEDURE PARAMETERS

Test: HCY*  
Type: Ser/Pl

ANALYZE

Assay time/Point  [2 Point End][10][19][34][0][0]
Wave (2nd/Primary)  [376][340]
S. Vol (Normal)  [16.5]
Reagent (R1) T1  [250][0] [000000]
Reagent (R2) T2  [0][0] [000000]
Reagent (R3) T3  [25][0] [000000]
Abs. Limit  [32000] [Decrease] 2 Tests
Prozone Limit  [32000] [0] [Lower]
Cell Detergent (Detergent 1)
CALIB
Calibration Type  [Linear]
Point  [2]
Span Point  [2]
Weight  [0]
Auto Calibration  
2 Point  [720]
SD Limit  [100]
Duplicate Limit  [10%] [32000 Abs]
Sensitivity Limit  [-99999] [99999]
S1 Abs limit  [32000] [32000]
RANGE
Application Code*  [ ] Unit [µmol/L]
Control Interval*  [ ]
Instrument Factor  [Y=aX+b] a=1.0 b=0.0
Technical Limit  [2.0] [46.0]
Repeat Limit*  [-99999] [99999]
OTHERS
<Standard>  (1) (2)
Calibration Code*  [ ] [ ]
Concentration**  [0.00] [**]
Position*  [ ] [1]
Sample Volume  [16.5] [16.5]

ROCHE INTEGRA 800 – PROCEDURE PARAMETERS

Test: HCY*  
Type: Ser/Pl

ANALYZE

Assay time/Point  [2 Point End][10][19][34][0][0]
Wave (2nd/Primary)  [376][340]
S. Vol (Normal)  [16.5]
Reagent (R1) T1  [250][0] [000000]
Reagent (R2) T2  [0][0] [000000]
Reagent (R3) T3  [25][0] [000000]
Abs. Limit  [32000] [Decrease] 2 Tests
Prozone Limit  [32000] [0] [Lower]
Cell Detergent (Detergent 1)
CALIB
Calibration Type  [Linear]
Point  [2]
Span Point  [2]
Weight  [0]
Auto Calibration  
2 Point  [720]
SD Limit  [100]
Duplicate Limit  [10%] [32000 Abs]
Sensitivity Limit  [-99999] [99999]
S1 Abs limit  [32000] [32000]
RANGE
Application Code*  [ ] Unit [µmol/L]
Control Interval*  [ ]
Instrument Factor  [Y=aX+b] a=1.0 b=0.0
Technical Limit  [2.0] [46.0]
Repeat Limit*  [-99999] [99999]
OTHERS
<Standard>  (1) (2)
Calibration Code*  [ ] [ ]
Concentration**  [0.00] [**]
Position*  [ ] [1]
Sample Volume  [16.5] [16.5]