Liquid reagents – ready to use

HOMOCYSTEINE
ENZYMATIC CYCLING
2 Reagents

Diagnostic reagent for the quantitative in vitro determination of total L-Homocysteine in human serum or plasma on photometric systems

REF | Cont. | 908500B | 780 ml | 600 ml | Reagent 1 | 180 ml | Reagent 2 |
908510 | 4 x 20 ml | 3 x 20 ml | 78 ml | 1 x 18 ml | Reagent 1 | 1 x 9 ml | Reagent 2 |
908520 | 4 x 10 ml | 3 x 10 ml | 39 ml | 1 x 9 ml | Reagent 1 | 1 x 9 ml | Reagent 2 |
992911 | 1 x 39 ml | 1 x 30 ml | 18 ml | 1 x 9 ml | Reagent 1 | 1 x 9 ml | Reagent 2 |
9A0849 | 4 x 20 ml | 3 x 20 ml | 78 ml | 1 x 18 ml | Reagent 1 | 1 x 18 ml | Reagent 2 |
9T1049 | 4 x 20 ml | 3 x 20 ml | 78 ml | 1 x 18 ml | Reagent 1 | 1 x 18 ml | Reagent 2 |

Additionally offered:
908550 | 5 x 1 ml | Homocysteine Calibrator Set (5 levels)
905620 | 1 x 1 ml | Homocysteine Control Set (4 levels)

TESTPARAMETER
Method: UV, 2 point kinetic (fixed time) reaction, enzymatic cycling
Wavelength: 340 nm
Temperature: 37 °C
Sample: Serum, heparin plasma, EDTA plasma
Linearity: up to 50 µmol/L
Sensitivity: The lower limit of detection is 0.4 µmol/L

SUMMARY
Elevated level of total Homocysteine (tHcy) has emerged as an important risk factor in the assessment of cardiovascular disease [1-3]. Excess homocysteine (Hcy) in the bloodstream may cause injuries to arterial vessels due to its irritant nature, and result in inflammation and plaque formation, which may eventually cause blockage of blood flow to the heart. Elevated levels of tHcy are also linked with Alzheimer’s disease [4] and osteoporosis [5].

TESTPRINCIPLE
Hcy + SAM > Hcy-methyltransferase > Methionine + SAH
SAH > SAH-hydrolase > Adenosine + Hcy
Adenosine > Adenosine deaminase > Inosine + NH3
NH3 + NADH + 2-Oxoglutarate > GLDH > Glutamate + NAD+ + H2O

In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM), catalyzed by a Hcy S-methyltransferase to form methionine (the Hcy conversion product of Hcy) and S-adenosylhomocysteine (SAH, the co-substrate conversion product). SAH is assessed by coupled enzyme reactions including SAH hydrolase, adenosine deaminase and glutamate dehydrogenase, wherein SAH is hydrolyzed into adenosine and Hcy by SAH hydrolase. The formed Hcy that is originated from the co-substrate SAM is cycled into the Hcy conversion reaction by Hcy S-methyltransferase. This forms a co-substrate conversion product based enzyme cycling reaction system with significant amplification of detection signals. The formed adenosine is immediately into inosine and ammonia. In the last step, the enzyme glutamate dehydrogenase (GLDH) catalyzes the reaction of ammonia with 2-oxoglutarate and NADH to form NAD+. The concentration of Hcy in the sample is directly proportional to the amount of NADH converted to NAD*.

REAGENT COMPOSITION
COMPONENTS | CONCENTRATION
S-Adenosylmethionine (SAM) | 0.1 mM
NADH | > 0.2 mM
TCEP | > 0.5 mM
2-Oxoglutarate | 5.0 mM
Glutamate dehydrogenase | 10 KU/L
SAH hydrolase | 3.0 KU/L
Adenosine deaminase | 5.0 KU/L
Hcy methyltransferase | 5.0 KU/L

REAGENT PREPARATION
The reagents are ready to use.

REAGENT STABILITY AND STORAGE
Conditions: Protect from light.
Close immediately after use. Avoid contamination.
Do not freeze!
Storage: at 2 – 8 °C
Stability: Up to the expiration date

SAMPLE COLLECTION AND HANDLING
Fresh serum, heparin plasma, or EDTA plasma can be used in the Homocysteine assay. Centrifuge blood sample immediately after collection! If immediate centrifugation is not possible, collected blood specimens should be kept on ice and centrifuged within an hour. Discard hemolysed, turbid or severely lipemic specimens.
Addition of 3-deazaadenosine to inhibit Hcy production in red cells has been suggested. However, the Dialab Hcy assay cannot use samples containing 3-deazaadenosine since it inhibits one of the key enzymes used in the assay.

SAMPLE STABILITY AND STORAGE
Serum, Heparin plasma or EDTA Plasma:
At room temperature: 4 days
at 0 – 8 °C several weeks
at -20 °C > 1 year

MATERIALS REQUIRED BUT NOT PROVIDED
Analyzer capable of measuring absorbance at 340 nm and temperature control
General laboratory equipment

ASSAY PROCEDURE
Main wavelength: 340 nm
Second wavelength: 700 nm
Sample: 13 µl
Reagent 1: 240 µl
Incubation Time: 5 minutes
Reagent 2: 65 µl
1st Reading: 2.5 minutes after adding R2
2nd Reading: 5 minutes after adding R2

REFERENCE RANGE
In most clinical laboratories, a concentration between 12 and 15 µmol/L is used as the cut-off value for normal level of Hcy for adults. However, each laboratory is recommended to establishes a range of normal values for the population in their region.

PERFORMANCE CHARACTERISTICS
LINEARITY, MEASURING RANGE
The test has been developed to determine homocysteine concentrations within a measuring range from 3 - 50 µmol/L. Samples with values greater than 50 µmol/L should be diluted 1:2 with distilled water and rerun. Multiply results by 2.

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 0.4 µmol/L.
**METHOD COMPARISON**

Correlation studies were performed by testing 40 serum samples in comparison with an existing commercial Hcy assay method. Linear regression gives the following equation:

\[ y = 0.94 \times + 1.05; R^2 = 0.99 \]

**PRECISION**

<table>
<thead>
<tr>
<th>Within run precision, n=20</th>
<th>Mean [µmol/L]</th>
<th>SD [µmol/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>7.08</td>
<td>0.33</td>
<td>4.61</td>
</tr>
<tr>
<td>Sample 2</td>
<td>12.4</td>
<td>0.24</td>
<td>1.91</td>
</tr>
<tr>
<td>Sample 3</td>
<td>16.2</td>
<td>0.51</td>
<td>3.12</td>
</tr>
<tr>
<td>Sample 4</td>
<td>26.9</td>
<td>0.66</td>
<td>2.47</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter run precision, n=30</th>
<th>Mean [µmol/L]</th>
<th>SD [µmol/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>7.32</td>
<td>0.44</td>
<td>5.98</td>
</tr>
<tr>
<td>Sample 2</td>
<td>11.3</td>
<td>0.57</td>
<td>5.08</td>
</tr>
<tr>
<td>Sample 3</td>
<td>14.4</td>
<td>0.81</td>
<td>5.61</td>
</tr>
<tr>
<td>Sample 4</td>
<td>27.7</td>
<td>0.72</td>
<td>2.61</td>
</tr>
</tbody>
</table>

**INTERFERING SUBSTANCES**

The following substances normally present in the serum produced less than 10% deviation when tested at levels equal to the concentrations listed below.

- Ascorbic Acid 10 mM
- Bilirubin 40 mg/dL
- Hemoglobin 500 mg/dL
- Triglyceride 1000 mg/dL
- Cystathionine 100 µM

Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticongivulants, or 6-azuridine triacetate may have higher levels of homocysteine due to metabolic interference with homocysteine metabolism. S-adenosylhomocysteine (SAH) will cause a significant positive interference. However, SAH is either not detectable or at very low concentrations in normal plasma and should not cause concern.

Automated chemistry analyzers use on-board routine wash steps to prevent reagent carry-over by reagent probes. However, the efficiency of the routine reagent probe wash varies and additional wash steps may be needed.

**CALIBRATION**

The assay requires the use of an Homocysteine Standard or Calibrator.

We recommend the Dialab Homocysteine Calibrator Set (5 levels).

**QUALITY CONTROL**

Homocysteine controls should be used to validate the performance of Hcy reagents.

We recommend the Dialab Homocysteine Control Set (4 levels).

Each laboratory should establish corrective action in case of deviations in control recovery.

**AUTOMATION**

Special adaptations for automated analyzers can be made on request.

**WARNINGS AND PRECAUTIONS**

1. For in vitro diagnostic use by suitably qualified laboratory personnel only
2. Do not ingest! Avoid contact with skin, eyes and mucous membranes.
3. 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.
4. Do not mix reagents of different lots.
5. Take the necessary precautions for the use of laboratory reagents.
6. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

**WASTE MANAGEMENT**

Please refer to local legal requirements.

**REFERENCES**