Liquid Reagents – ready to use

**HOMOCYSTEINE**

**ENZYMATIC CYCLING**

2 Reagents

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

### REAGENT COMPOSITION

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>FINAL CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Adenosylmethionine (SAM)</td>
<td>0.1 mM</td>
</tr>
<tr>
<td>NADH</td>
<td>&gt; 0.2 mM</td>
</tr>
<tr>
<td>TCEP</td>
<td>&gt; 0.5 mM</td>
</tr>
<tr>
<td>2-Oxoglutarate</td>
<td>5.0 mM</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>10 KU/L</td>
</tr>
<tr>
<td>SAH hydrolase</td>
<td>3.0 KU/L</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>5.0 KU/L</td>
</tr>
<tr>
<td>Hcy methyltransferase</td>
<td>5.0 KU/L</td>
</tr>
</tbody>
</table>

### REAGENT PREPARATION

The reagents are ready to use.

### REAGENT STABILITY AND STORAGE

- Conditions: protect from light
- Close immediately after use
- Do not freeze!
- Storage: at 2 – 8°C

The reagent should be clear. It should be discarded if it becomes turbid or the initial absorbance is less than 0.5 at 340 nm (light path 0.6 cm).

### SAMPLE COLLECTION AND HANDLING

Fresh serum, heparin plasma, or EDTA plasma can be used in the Homocysteine assay.

Centrifuge blood samples 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. Additional 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

- NaCl solution (9 g/L)
- General laboratory equipment

### INTERFERING SUBSTANCES

Patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azauridine triacetaate may have higher levels of homocysteine due to metabolic interference with homocysteine metabolism.

### TEST PRINCIPLE

\[
\text{Hcy + SAM} \rightarrow \text{Hcy-methyltransferase} \rightarrow \text{Methionine + SAH} \\
\text{SAH} \rightarrow \text{SAH-hydrolase} \rightarrow \text{Adenosine + Hcy} \\
\text{Adenosine} \rightarrow \text{Adenosine deaminase} \rightarrow \text{Inosine + NH}_3 \\
\text{NH}_3 + \text{NADH} + 2-\text{Oxoglutarate} \rightarrow \text{GLDH} \rightarrow \text{Glutamate + NAD}^+ + \text{H}_2\text{O}
\]

In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, SAM, catalyzed by a Hcy S-methyltransferase to form methionine (the Hcy conversion product of Hcy) and 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 hydrolyzed into Inosine and ammonia which reacts with glutamate dehydrogenase with concomitant conversions of NADH to NAD⁺. The concentration of Hcy in the sample is proportional to the amount of NADH converted to NAD⁺.

**ABBREVIATIONS**

Hcy = Homocysteine  
SAM = S-adenosylmethionine  
SAH = S-adenosylhomocysteine  
GLDH = Glutamate dehydrogenase

**PERFORMANCE CHARACTERISTICS**

**LINEARITY, SENSITIVITY**

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 water and rerun. Multiply results by 2.

**ACCURACY**

Correlation studies were performed by testing 32 patient samples in comparison with another commercial Hcy assay method. The linear regression gives the following equation:

\[ y = 0.93 \times x + 1.39; R^2 = 0.97 \]

**PRECISION (at 37°C)**

<table>
<thead>
<tr>
<th></th>
<th>Mean [μmol/L]</th>
<th>SD [μmol/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within run precision, n=20</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>

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<thead>
<tr>
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<th>SD [μmol/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inter run precision, n=30</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>

**QUALITY CONTROL**

We recommend that each laboratory uses Hcy controls to validate the performance of Hcy reagents.

We recommend:

- **REF 905620**  
  4 x 1 ml  
  Homocysteine Control Set  
  (4 levels)

**CALIBRATION**

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

We recommend:

- **REF 908550**  
  5 x 1 ml  
  Homocysteine Calibrator Set  
  (5 levels)

**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. Take the necessary precautions for the use of laboratory reagents.
5. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

**REFERENCES**


**WASTE MANAGEMENT**

Please refer to local legal requirements.