**Cholinesterase Reagent**

### PRODUCT SUMMARY

**Stability**: 3 days at 2-8°C  
**Linear Range**: Up to 8000 U/L  
**Specimen Type**: Serum  
**Method**: Kinetic  
**Reagent Preparation**: Add specified volume of distilled or deionized water.

### SYMBOLES IN PRODUCT LABELLING

- **EC REP**: Authorized Representative  
- **LVD**: For in vitro diagnostic use  
- **LOT**: Batch code/Lot number  
- **REF**: Catalogue number  
- **Temperature Limitation**: Use by/Expiration Date  
- **CAUTION**: Consult instructions for use  
- **Manufactured by**: For use.

### INTENDED USE

This reagent is intended for the in vitro quantitative determination of Cholinesterase in human serum.

### CLINICAL SIGNIFICANCE

There are two forms of cholinesterase: acetyl cholinesterase and cholinesterase or also commonly referred to as pseudocholinesterase. Acetylcholinesterase is found predominantly in erythrocytes. Cholinesterase is synthesised in the liver and is present in plasma and is the form of the enzyme routinely measured. Cholinesterase is most commonly measured as an indicator of exposure to anticholinesterases (organophosphates, including many insecticides), or inherited abnormal variants of the enzyme, which cause a decreased level of plasma cholinesterase. Increased levels of activity may be present in nephrotic syndrome or in the recovery from liver damage.

### METHODOLOGY

Cholinesterase hydrolyses propionylthiocholine to propionic acid and thiocholine. Thiocoline reacts with 5,5'-dithio-bis (2-nitrobenzoic acid) to form the yellow coloured 5-thio-2-nitrobenzoic acid. The rate of formation of 5-thio-2-nitrobenzoic acid, measured at 405nm, is directly proportional to cholinesterase activity in the sample. This method is a modification of the methodology of Dietz et al.

\[
\text{Reaction:} \quad \text{Cholinesterase} + \text{Propionylthiocholine} \rightarrow \text{Propionic acid} + \text{Thiocholine}
\]

### REAGENT COMPOSITION

- **Active Ingredients**: Concentration  
  - Propionylthiocholine iodide: 4.0 mmol/L  
  - 5,5'-Dithio-bis-(2-nitrobenzoic acid): 0.25 mmol/L  
- **Buffer**: pH 6.8

### REAGENT PREPARATION

Reconstitute the contents of each vial with the volume of distilled or deionised water stated on the vial label. Mix gently until fully dissolved. DO NOT SHAKE.

### STABILITY AND STORAGE

**Prior to use**:  
When stored between 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

**Reconstituted Reagent**:  
When stored capped at 2-8°C, the reagent is stable for at least 3 days.

**Indications of Reagent Deterioration**:  
- Turbidity,  
- Absorbance > 0.8 at 405nm (1cm); and/or  
- Failure to recover control values within the assigned range.

### SPECIMEN COLLECTION AND HANDLING

- **Serum**: Use non-haemolysed serum.  
- **Storage**: Cholinesterase in serum is stable for 17 days when stored between 4-23°C or for 3 months when stored below -20°C.

### CALCULATIONS

**Activity in U/L = ΔAbs/min x Factor**

**Factor = TV x 1000 x 2 / 14.64 x SV x P**

Where:  
- **TV** = Total reaction volume in mL  
- **SV** = Sample volume in mL  
- **14.64** = Millimolar absorption coefficient of 5-thio-2-nitrobenzoic acid at 405nm (see Note 3).  
- **P** = Cuvette pathlength in cm  
- **2** = Conversion from ΔAbs/30sec to ΔAbs/min

**Example**:  
- **ΔAbs/30sec** = 0.150  
- **Factor** = 13,798  
- **Cholinesterase** = 0.150 x 13,798 = 2070 U/L

### NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.  
2. Valid results depend on an accurately calibrated instrument, timing, and temperature control.  
3. The millimolar absorption coefficient for 5-thio-2-nitrobenzoic acid at 405 nm is 14.64.  
4. **Unit Conversion**: U/L x 16.67 x 10^-3 = µkat/L

### CALIBRATION

Not required. The rate of reaction is converted to U/L of activity by a calculation factor. Refer to the calculation section of this package insert.
QUALITY CONTROL
To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-
- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control. The following corrective actions are recommended in such situations: -
- Repeat the sample controls
- If repeated results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat test with fresh reagent.
- If results are still out of control contact Technical Services or your local distributor.

LIMITATIONS
1. Young DS has published a comprehensive list of drugs and substances which may interfere with this assay.
2. Grossly haemolysed samples may produce falsely elevated results.
3. Avoid lipaemic and icteric samples.

EXPECTED VALUES
At 30°C  2618 - 6971 U/L (43.6 - 116.2 µkat/L)
The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.

PERFORMANCE DATA
The following data was obtained using the Cholinesterase Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION
Within Run:

<table>
<thead>
<tr>
<th>Level</th>
<th>Number of samples</th>
<th>Mean (U/L)</th>
<th>SD (U/L)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20</td>
<td>1021</td>
<td>14.9</td>
<td>1.5</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>3845</td>
<td>44.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Total:

<table>
<thead>
<tr>
<th>Level</th>
<th>Number of samples</th>
<th>Mean (U/L)</th>
<th>SD (U/L)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20</td>
<td>1021</td>
<td>24.3</td>
<td>2.4</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>3845</td>
<td>81.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

ACCURACY
Comparison studies were carried out using a similar commercially available Cholinesterase reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sample pairs</td>
<td>80</td>
</tr>
<tr>
<td>Range of sample results</td>
<td>556 - 6581 U/L</td>
</tr>
<tr>
<td>Slope</td>
<td>0.909</td>
</tr>
<tr>
<td>Intercept</td>
<td>15.8 U/L</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9819</td>
</tr>
</tbody>
</table>

LINEARITY:
When run as recommended the assay is linear up to 8000 U/L (133.4 µkat/L).

SENSITIVITY:
When run as recommended the sensitivity of the assay is 0.072 nmol/min per U/L.

REFERENCES