CHOLINESTERASE TOTAL AND INHIBITED
Enzymatic colorimetric method
KINETIC

CHOLINESTERASE

Butyrylthiocholine + H2O \[\text{CHE}\] \rightarrow \text{Butyrate} + \text{Thiocholine}
Thiocholine + DMNB \[\rightarrow\] Oxidized thiocholine + 5-MNBA + H²

Dibucaine inhibition can be estimated by performing concurrent assays in which dibucaine is present in the substrate mixture. Percent inhibition is evaluated by comparison of activity in the inhibited system with that in the uninhibited system. The resulting dibucaine number allows the classification and identity of the homozygous and heterozygous variants.

REAGENT COMPOSITION

R1 Buffer/Chromogen. Phosphate buffer 50 mmol/L pH 7.7, DMNB 0.25 mmol/L. Powder.
R2 Substrate. Butyrylthiocholine iodide 7 mmol/L. Freeze-dried.
R3 Dibucaine. Dibucaine Chloridrate 2.6 mmol/L.

STORAGE AND STABILITY

Store at 2-8°C. All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date. Store the vials tightly closed, protected from light and prevented contaminations during the use.

Discard if appear signs of deterioration:
- Presence of particles and turbidity.
- Discard reconstituted reagents R1 and R2 if present an Blank absorbance (A) at 405 nm > 0.700 in 1 cm cuvette against distilled water.

REAGENT PREPARATION

Working reagents.
1. **Buffer/Chromogen.** Add 25 mL of distilled water into a vial of R1. Cap. Shake. Stand for 15 min. before use. Stable for 6 weeks at 2-8°C.
2. **Substrate.** Add 2.0 mL of distilled water into a vial of R2. Mix. Stable for 6 weeks at 2-8°C. Excess substrate may be frozen once.
3. **Inhibitor reagent.** Mix 9 volumes of Buffer/Chromogen with 1 volumen of R3.

SAMPLES

Serum, EDTA or heparinized plasma. Moderate hemolysis does not interfere.
Cholinesterase in serum or plasma is stable for several weeks whether the specimen is stored at room temperature or under refrigeration, and for 3 months at –20°C.

INTERFERENCES

- Lipemia (intranalipid).20 g/L does not interfere.
- Bilirubin, 40 mg/dL does not interfere.
- Hemoglobin, 16 g/L does not interfere.
- Other drugs and substances may interfere.

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 25/30/37°C, capable to read at 405 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Preincubate working reagents and samples to reaction temperature (see NOTES).
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into labelled cuvettes:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Buffer/Chromogen</th>
<th>Inhibitor reagent</th>
<th>Sample</th>
<th>Sample di 1:2 with saline</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>25/30°C</td>
<td>1.5 mL</td>
<td>–</td>
<td>10 µL</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>37°C</td>
<td>1.5 mL</td>
<td>1.5 mL</td>
<td>10 µL</td>
<td>10 µL</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

4. Mix gently by inversion. Insert cuvette into the cell holder, start stopwatch and record the initial absorbance.
5. Repeat the absorbance readings exactly after 30, 60 and 90 seconds.
6. Calculate the difference between absorbances.
7. Calculate the mean of the results to obtain the average change in absorbance per second (ΔA/30 sec).
**CALCULATIONS**

**Total cholinesterase**

\[
\text{U/L} = \Delta A/ \text{1 min x } 23111 \quad (37^\circ C)
\]

\[
\text{U/L} = \Delta A/ \text{30 sec x } 46222 \quad (37^\circ C)
\]

\[
\text{U/L} = \Delta A/ \text{30 sec x } 23111 \quad (25/30^\circ C)
\]

Samples with \(\Delta A\) exceeding 0.250 at 405 nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

If results are to be expressed as SI units apply:

\[
\text{U/L} = \text{nmol/min/L x 0.1}
\]

**Inhibited cholinesterase**

To express the dibucaine number apply:

\[
\text{Percent inhibition} = \left(1 - \frac{\text{U/mL with inhibitor}}{\text{U/mL without inhibitor}}\right) \times 100
\]

**REFERENCE VALUES**

**Total cholinesterase**

Serum, plasma

<table>
<thead>
<tr>
<th>Age</th>
<th>Activity (KU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children, Males and females &gt; (40 years)</td>
<td>3.5-6.5 KU/L (58.3-141.7 µktal/L)</td>
</tr>
<tr>
<td>Females, (16-39 years) Nonpregnant, not taking contraceptives</td>
<td>2.8-7.4 KU/L (46.7-123.3 µktal/L)</td>
</tr>
<tr>
<td>Females, (18-41 years) Pregnant or taking contraceptives</td>
<td>2.4-6.0 KU/L (40.0-100.0 µktal/L)</td>
</tr>
</tbody>
</table>

**Inhibited cholinesterase**

<table>
<thead>
<tr>
<th>Type</th>
<th>Dibucaine number</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal homozygous</td>
<td></td>
<td>70-90</td>
</tr>
<tr>
<td>Heterozygous</td>
<td></td>
<td>35-75</td>
</tr>
<tr>
<td>Atypical homozygous</td>
<td></td>
<td>0-20</td>
</tr>
</tbody>
</table>

**QUALITY CONTROL**

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

**REFERENCES**


**NOTES**

1. Buffer/Chromogen and Substrate can be mixed proportionally in tests or analysers using the serum as starter. The mixture is stable for 2 hours at 15-25ºC.
2. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
3. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

**ANALYTICAL PERFORMANCE**

- **Detection Limit** : 244 U/L
- **Linearity** : Up to 10000 U/L
- **Precision**:

<table>
<thead>
<tr>
<th></th>
<th>U/L</th>
<th>Within-run</th>
<th>Between-run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.11</td>
<td>8.01</td>
<td>5.11</td>
</tr>
<tr>
<td>SD</td>
<td>0.04</td>
<td>0.106</td>
<td>0.091</td>
</tr>
<tr>
<td>CV%</td>
<td>0.79</td>
<td>1.36</td>
<td>1.78</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

- **Sensitivity** : 0.08 mA / U/L cholinesterase.
- **Correlation**: This assay (y) was compared with a similar commercial method (x). The results were:

\[
N = 50 \quad r = 0.995 \quad y = 0.987 x + 0.005
\]

The analytical performances have been generated using on automatic instrument. Results may vary depending on the instrument.

**REFERENCES**