KALLIKREIN INHIBITOR ACTIVITY

**Determination of kallikrein inhibitor activity in plasma, with S-2302.**

**Measurement Principle**

Plasma is incubated with a purified plasma kallikrein preparation. The amount of kallikrein inhibited is proportional to the activity of the kallikrein inhibitor present in the plasma. The remaining amount of kallikrein activity is then determined by using the substrate H-D-Pro-Phe-Arg-pNA (S-2302). The rate at which p-nitroaniline (pNA) is released is measured photometrically at 405 nm. This can be followed on a recorder (initial rate method) or read after stopping the reaction with acetic acid (acid stopped method).

\[
K-\text{Inhibitor} + K \text{ (excess)} \rightarrow [K-\text{Inhibitor} \cdot K] + K \text{ (residual)}
\]

\[
H-D\text{-Pro-Phe-Arg-pNA} + H_2O \rightarrow H-D\text{-Pro-Phe-Arg-OH} + pNA
\]

**Reagents**

   Reconstitute the substrate S-2302 (MW: 611.6) with 20 ml of distilled water.
2. Plasma Kallikrein
   Use purified human plasma kallikrein (refer to Gallimore MJ et al., 1978). Prepare a solution of 1 nkat S-2302/ml human plasma kallikrein in Tris buffer pH 7.8. 1 nkat S-2302 corresponds to 0.06 U or 0.017 PEU (refer to Friberger P et al. 1979).
3. Tris Buffer, pH 7.8 (25°C)
   Tris 6.1 g (50 mmol/l)
   NaCl 21.1 g (361 mmol/l)
   Polybrene 20 mg
   Distilled water 800 ml
   Adjust the pH to 7.8 at 25°C by adding an appropriate amount (approx. 38 ml) of 1 mol/l HCl. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, will remain stable for six months at 2 to 8°C.
4. Normal plasma
   Blood samples are taken from at least 10 healthy donors. For the preparation of the samples, refer to the Specimen collection section.
5. Acetic acid, 20%
   Acetic acid is used in the acid stopped method.

**Equipment**

1. Spectro- or filter photometer, 405 nm
2. Siliconised semi-microcuvettes, 1 cm
3. Centrifuge
4. Thermostat, 37°C
5. Stop watch
6. Disposable plastic tubes

**Specimen collection**

Blood (9 vol) is mixed with 0.1 mol/l sodium citrate (1 vol) and centrifuged at 2000 x g for 20 minutes at 15-25°C. In order to avoid low-temperature activation of plasma kallikrein inhibitor the plasma should be kept at 15-25°C for not more than a few hours or immediately frozen at -20°C or below. After thawing at 37°C the plasma should be kept at 15-25°C and used as soon as possible. Frozen plasma may lose some plasma kallikrein inhibitor activity on freezing or thawing, but is stable for several months at -20°C or below.

**Standard curve**

Normal plasma has a kallikrein inhibitor concentration of 100% and is diluted according to the table below (see Note 1).

<table>
<thead>
<tr>
<th>%</th>
<th>Normal Plasma µl</th>
<th>Buffer µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>50</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>75</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>400</td>
<td>-</td>
</tr>
</tbody>
</table>

**Method**

**Sample dilution**

<table>
<thead>
<tr>
<th>Tube No. 1</th>
<th>Buffer</th>
<th>Test Plasma or Standard (see note 1)</th>
<th>Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1900 µl</td>
<td>100 µl</td>
<td></td>
</tr>
</tbody>
</table>

**Initial rate method**

**Tube No. 2**

<table>
<thead>
<tr>
<th>Sample from tube No. 1</th>
<th>200 µl</th>
<th>Incubate at 37°C</th>
<th>3-4 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma kallikrein</td>
<td>200 µl</td>
<td>Mix</td>
<td></td>
</tr>
<tr>
<td>Substrate (37°C)</td>
<td>200 µl</td>
<td>Mix</td>
<td></td>
</tr>
</tbody>
</table>

Transfer sample immediately to a 1 cm siliconised semi-microcuvette (preheated to 37°C) for measurement of the absorbance change in a photometer at 405 nm and at 37°C. Calculate ∆A/min.

**Acid stopped method**

**Tube No. 2**

<table>
<thead>
<tr>
<th>Sample from tube No. 1</th>
<th>200 µl</th>
<th>Incubate at 37°C</th>
<th>3-4 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma kallikrein</td>
<td>200 µl</td>
<td>Mix</td>
<td></td>
</tr>
<tr>
<td>Substrate (37°C)</td>
<td>200 µl</td>
<td>Mix</td>
<td></td>
</tr>
</tbody>
</table>

| Acetic acid 20%         | 200 µl | Mix              |         |
Plasma blanks are prepared by adding the reagents in reverse order without incubation. Read the absorbance (A) of the sample against its blank in a photometer at 405 nm. The colour is stable for at least 4 hours.

**Calculation**

**Plasma Kallikrein inhibitor in percentage of normal plasma**
Plot A or \( \Delta A/min \) for the standards against their concentration of kallikrein inhibitor on log-lin graph paper. Read the kallikrein inhibitor value for the corresponding A or \( \Delta A/min \) of the unknown test sample from the standard curve.

**Plasma Kallikrein inhibitor in enzyme activity units**
In each test series a kallikrein activity determination with buffer instead of sample dilution must be performed. The difference between this activity and the sample activity is then calculated.

- **Initial rate method:**
  \[
  \mu\text{kat/l} = (\Delta A/\text{min buffer} - \Delta A/\text{min sample}) \times 104
  \]
  \[
  \mu\text{l/l} = (\Delta A/\text{min buffer} - \Delta A/\text{min sample}) \times 6250
  \]

- **Acid stopped method:**
  \[
  \mu\text{kat/l} = (A_{\text{buffer}} - A_{\text{sample}}) \times 34.7
  \]
  \[
  \mu\text{l/l} = (A_{\text{buffer}} - A_{\text{sample}}) \times 2080
  \]

**Notes**
1. A 150% standard is prepared by diluting 300 µl normal plasma with 3700 µl buffer. A 200% standard is prepared by diluting 100 µl normal plasma with 900 µl buffer. For 0% use the buffer only (note that the absorbance to surfaces can result in lower readings when plasma is absent).
2. It is suggested, that the spontaneous kallikrein activity (\( \alpha_2\)-M complex) should be determined in patients in whom the kallikrein system is suspected to be activated.

**Bibliography**


