**Chymotrypsin**

**Determination of chymotrypsin activity with S-2586**

**Measurement Principle**

The chymotrypsin activity is determined by its amidolytic effect on the substrate MeO-Suc-Arg-Pro-Tyr-pNA (S-2586). 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). The correlation between the change in absorbance per minute (DA/min) or absorbance (A) and the chymotrypsin activity is linear in the 0.05-1.0 µkat/l or 3-60 U/l range. The amidolytic activity of different chymotrypsin preparations does not necessarily parallel the protease activity.

\[
\text{MeO-Suc-Arg-Pro-Tyr-pNA} + \text{H}_2\text{O} \quad \xrightarrow{\text{Chymotrypsin}} \quad \text{MeO-Suc-Arg-Pro-Tyr-OH} + \text{pNA}
\]

**Reagents**

1. **S-2586**, 25 mg   
   Art. No. 82 08 94  
   Reconstitute the substrate S-2586 (MW: 705.3) with 60 ml of distilled water.
2. **Buffer - Tris/CaCl**, pH 8.3 (25°C)
3. **Tris** 12.1 g  
   (100 mmol/l)  
   **NaCl** 56.2 g  
   (960 mmol/l)  
   **Distilled water** 800 ml

Adjust the pH to 8.3 at 25°C by adding approximately 50 ml of 1 mol/l HCl. Add 10 ml of 1 mol/l CaCl 2 solution. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, will remain stable for two months at 2-8°C.

4. **Acetic acid 20%**  
   Acetic acid is used in the acid-stopped method.

**Sample**

The sample containing chymotrypsin is dissolved in or diluted with 1 mmol/l HCl to a concentration of 0.1 g/l. This stock solution is stable for more than two weeks at 2-8°C. Before assay, the solution is diluted 1:200 with 1 mmol/l HCl. If the sample is a pure protein, it is advisable to use 0.1% Carbowax 6000 (Union Carbide, NY) or 1% albumin (previously checked for amidolytic activity) to avoid adsorption to surfaces.

**Method**

<table>
<thead>
<tr>
<th>Initial rate method</th>
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<tbody>
<tr>
<td><strong>Buffer</strong> 200 µl</td>
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<tr>
<td><strong>Incubate at 37°C</strong> 3-4 min</td>
</tr>
<tr>
<td><strong>Chymotrypsin sample</strong> 200 µl</td>
</tr>
<tr>
<td><strong>Mix and incubate at 37°C</strong> 2-3 min</td>
</tr>
<tr>
<td><strong>Substrate</strong> 200 µl</td>
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</tbody>
</table>

Transfer immediately to a 1 cm 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.
method | Sample | Blank
--- | --- | ---
Buffer | 200 ml | 200 µl
Incubate at 37°C | 3-4 min | -
Chymotrypsin sample | 200 ml | 200 µl
Mix and incubate at 37°C | 2-3 min | -
Substrate (37°C) | 200 ml | -
Mix and incubate at 37°C | 3 min | -
Acetic acid 20% | 200 ml | 200 µl
Mix | yes | -
Substrate | - | 200 µl
Mix | - | yes

Read the absorbance (A) of the sample against distilled water at 405 nm within 4 hours.

**Calculation**

Calculate the chymotrypsin activity of the stock solution from the following formulas:

Initial rate method:

\[
\text{µkat/l} = 5.19 \times \Delta A/\text{min} \times 200
\]

\[
\text{U/l} = 311 \times \Delta A/\text{min} \times 200
\]

Acid stopped method:

\[
\text{µkat/l} = 2.31 \times A \times 200
\]

\[
\text{U/l} = 138 \times A \times 200
\]

**Bibliography**