**Product overview**

**Composition**
Solution in 50% glycerol, pH approx. 6.5

**pH optimum**
The optimum value is 6.0–6.5; activity reduces above or below this range.

**Temperature optimum**
Remains completely stable for 18 h; at +48°C it loses 35% of its original activity within 2 h.

**Application**
- Hydrolysis of steroid conjugates (glucuronides) in urine (pH 6.0–6.5) (3, 10).
- Drug analysis (6, 7, 8, 9)
- Detection of benzodiazepine in small doses (4)
  1) In case of determination of pregnanediol together with estrogens, use hydrolysis time given with "estrogens (total)"
  2) Then extraction of the free steroids and sulfate conjugates with acetic acid ethylester and following solvolyse (18 h; +38°C) of the sulfate conjugates.

<table>
<thead>
<tr>
<th>Compound or category</th>
<th>Parts in sample</th>
<th>Enzyme solution: drops</th>
<th>Hydrolysis: °C/min</th>
<th>Method of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Hydroxy-cortico-steroids</td>
<td>5</td>
<td>1</td>
<td>37°C/75 42°C/60 46°C/45</td>
<td>Porter &amp; Silber's method</td>
</tr>
<tr>
<td>Estrogens (total)</td>
<td>5–50 (vary with concentration)</td>
<td>1 per 5 ml</td>
<td>37°C/75 42°C/60 46°C/45</td>
<td>Kober's colorimetric or Ittrich's fluorimetric method</td>
</tr>
<tr>
<td>Pregnanediol</td>
<td>5–50 (vary with concentration)</td>
<td>1 per 5 ml</td>
<td>37°C/40 42°C/30 46°C/20</td>
<td>Talbot's colorimetric method, thin-layer chromatography, or gas chromatography</td>
</tr>
<tr>
<td>Estriol (in pregnancy)</td>
<td>5–10</td>
<td>2 per 5 ml</td>
<td>37°C/40 42°C/30 46°C/20</td>
<td>Kober's colorimetric or Ittrich's fluorimetric method, or gas chromatography</td>
</tr>
<tr>
<td>Pregnanetriol</td>
<td>50</td>
<td>10</td>
<td>37°C/75 42°C/60 46°C/45</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>Estradiol, estrene, estriol</td>
<td>5–50</td>
<td>1 per 5 ml</td>
<td>37°C/75 42°C/60 46°C/45</td>
<td>Separate compounds by gas chromatography; Baud's modification of Cohen &amp; Marrian's method if only the estrogen coefficient is needed</td>
</tr>
<tr>
<td>17-Keto-steroids</td>
<td>50</td>
<td>10</td>
<td>37°C/75 42°C/60 46°C/45</td>
<td>Gas chromatography or chromatography in liquid phase</td>
</tr>
</tbody>
</table>

**Principle of the standard test**

4-nitrophenyl-D-β-glucuronide $\rightarrow$ β-glucuronidase $\rightarrow$ 4-nitrophenol + glucuronic acid

**Steroids in urine**
The various steroids found in urine may be present in one or more of three forms:

a) the free compound (in minor or trace quantities and of little importance);

b) the sulfate (may be predominant in some cases);

c) the β-glucuronide (the predominant form in most cases).

**Storage/ Stability**
The solution is stable at +2 to +8°C until the expiration date printed on the label; storage at -15 to -25°C might well prolong the life of the preparation, but this has not been tested.

**Note:** During the first 6 months, the loss of activity may reach about 10%.
Advantages

In the following table the advantages of β-glucuronidase derived from *E. coli* are listed.

<table>
<thead>
<tr>
<th>Benefit</th>
<th>Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>High specific activity</td>
<td>Quantitative hydrolysis of steroid compounds in very short time (only 15–50 min, depending on enzyme concentration).</td>
</tr>
<tr>
<td>Great affinity for various β-glucuronides</td>
<td>It is particularly useful for hydrolysing steroid β-glucuronides in urine, since it displays great affinity for various β-glucuronides whose concentrations in urine are likely to be small.</td>
</tr>
<tr>
<td>No need for cleaning-up procedures after hydrolysis</td>
<td>During the course of hydrolysis of steroid β-glucuronides, it releases very little of the non-specific chromogens occurring in urine.</td>
</tr>
<tr>
<td>No need for buffering urine</td>
<td>Provided the pH value is adjusted to 6.0–6.5, since β-glucuronidase derived from <em>E. coli</em> is relatively indifferent to the ionic milieu.</td>
</tr>
</tbody>
</table>

Specific activity of Glucuronidase

The international unit of β-glucuronidase activity is the enzyme activity that increases the rate of release of 4-nitrophenol from 4-nitrophenyl-β-D-glucuronic acid (4NPG) at a temperature of +25°C and pH 7.0 by 1 μM. The Fishman unit was used formerly (5). This is defined in terms of the release of phenolphthalein from its glucuronide (PPG). However, it is not possible to measure the relative activities of different preparations with respect to steroid β-glucuronides just by comparing their activities with respect to PPG. Various kinds of preparation do not catalyse the hydrolysis of PPG, 4NPG, or the various steroid β-glucuronides in urine equally well. The choice of 4NPG as standard substrate was based on the following considerations:

(a) although the Michaelis concentrations for the two substrates are not very dissimilar (KM = 2 × 10⁻⁸ M for 4NPG and KM = 6 × 10⁻⁹ M for PPG), the corresponding rates of hydrolysis differ more: 4NPG is hydrolysed about 5 × as fast as PPG;
(b) in the case of PPG, inhibition through excess substrate is observed; this does not occur with 4NPG.

Specific activity

With 4-nitrophenyl-β-D-glucuronide (4NPG) as substrate, the specific activity of β-glucuronidase is approx. 140 U/mg at 37°C or 80 U/mg at +25°C.

The strength of the solution at +37°C is at least 140 U/ml.

References


Ordering Information

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Glucuronidase/ Arylsulfatase</td>
<td>2 ml</td>
<td>10 127 060 001</td>
</tr>
</tbody>
</table>

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To ask questions, solve problems, suggest enhancements and report new applications, please visit our Online Technical Support Site.

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