**β-Glucuronidase/Arylsulfatase**

obtained from *Helix pomatia*  
β-D-Glucuronoside glucuronosohydrolase, EC 3.2.1.31  
Aryl-sulfate sulfohydrolase, EC 3.1.6.1

Cat. No. 10 127 060 001 2 ml  
Cat. No. 10 127 698 001 10 ml

**Product overview**

**Composition**  
Enzyme mix of β-glucuronidase/arylsulfatase obtained from *Helix pomatia* in saline, stabilized with 0.02% sodium azide.

**pH optimum**  
• The pH optimum value for β-glucuronidase activity is 4.5–5.0.  
• The optimum for arylsulfatase activity is generally 6.2, but may be greater for some substrates in comparatively high concentrations; for instance, for 16.5 mM solutions of 2-hydroxy-5-nitrophenylhydrogen sulfate it is 7.2.

**Inhibitors**  
• β-Glucuronidase activity is inhibited by:  
  - D-glucuronic acid  
  - D-galacturonic acid  
  - D-glucaro-1,4-lactone (saccharolactone found in urine)  
• Arylsulfatase activity is inhibited by phosphate.

**Application**  
The enzyme preparation obtained from the Roman snail, *Helix pomatia*, which exhibits very strong β-glucuronidase and arylsulfatase activity, is widely used for the simultaneous hydrolysis of β-glucuronides (β-glucosiduronic acids) and sulfate esters in urine and other biological fluids (1, 2, 3, 4).  
• Enzymatic hydrolysis of steroid β-glucuronides and sulfates  
• Cell biology (removal of cell walls from yeasts in the preparation of protoplasts) (5)  
• Enzyme immobilization studies (6)  
• Determination of drugs in urine (7)

**Storage/ Stability**  
Undiluted, the aqueous solution of β-glucuronidase/arylsulfatase is stable at +2 to +8°C until the expiration date printed on the label.  
**Note:** Aliquots portions of the diluted preparation may be stored at -15 to -25°C; they should not be thawed and refrozen more than once or twice; and storage at lower temperatures does not lengthen their expiration date beyond that of the product kept at +2 to +8°C.

**Working concentration**  
In many applications the product can be diluted with water immediately before use or used undiluted.  
**Note:** This β-glucuronidase/arylsulfatase preparation is very concentrated and must be diluted for some applications. In particular for the preparation of protoplasts, the precise concentration to use for a given strain of yeast must be determined empirically.

**Product description**

**Specificity of β-Glucuronidase**  
The glycosides that β-D-glucuronic acid forms with a variety of compounds containing hydroxyl groups, hydrolyse readily in the presence of β-glucuronidase. Such compounds include:  
• steroids, such as estriol (Kₘ = 0.42 mM, pH 4.5), androsterone, pregnanediol, tetrahydrocortisone  
• phenols, such as phenolphthalein (Kₘ = 0.39 mM).  
• 4-nitrophenol, 4-methylumbelliferone  
• drugs, such as chloramphenicol and tetrahydrocanabinols  
• metabolites, such as thyroxine and bilirubin  
Polysaccharides that contain β-glucuronic acid residues, such as hyaluronic acid, are also hydrolyzed.  
β-Glucuronidase is highly specific for the carbohydrate moiety; neither α-glucosides nor β-glucosiduronic acids are hydrolyzed.

**Specificity of Arylsulfatase**  
Sulfate esters of many phenols are hydrolyzed in the presence of arylsulfatase. Examples are steroid sulfates, such as estronesulfate, 4-nitrophenyl hydrogen sulfate (Kₘ = 1.8 mM, pH 7.3), 4-nitro-pyrocatechol 2-sulfate (Kₘ = 1.25 mM, pH 7.5), and phenolphthalein disulfate.

**Principle of the standard test**  
At a wavelength of 405 nm, the molar absorption coefficient of 4-nitrophenol is 1.85 mM⁻¹ × l × cm⁻¹ at +25°C.

**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 amounts)  
(b) the sulfate (predominant in some cases)  
(c) the β-glucuronide (the predominant form in most cases)
Please refer to the following table.

<table>
<thead>
<tr>
<th>Compound or category</th>
<th>free [%]</th>
<th>sulfate [%]</th>
<th>glucuronide [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Hydroxycorticosteroids</td>
<td>10–15</td>
<td>85–90</td>
<td></td>
</tr>
<tr>
<td>Pregnaneol</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Pregnanetriol</td>
<td>trace</td>
<td>~100</td>
<td></td>
</tr>
<tr>
<td>Estrone (O₁)</td>
<td>1–3</td>
<td>5–10</td>
<td>90–95</td>
</tr>
<tr>
<td>Estradiol (O₂)</td>
<td>0–2</td>
<td>5–10</td>
<td>90–95</td>
</tr>
<tr>
<td>Androsterone</td>
<td>trace</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Etocholestanolone</td>
<td>trace</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Dihydroepiandrosterone (DHEA)</td>
<td>trace</td>
<td>~100</td>
<td>trace</td>
</tr>
<tr>
<td>Epiandrosterone</td>
<td>trace</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>11β-Androsterone</td>
<td>trace</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>11β-Etocholestanolone</td>
<td>trace</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>11 Ketandrosterone</td>
<td>trace</td>
<td>trace</td>
<td>~100</td>
</tr>
<tr>
<td>11 Ketotocholestanolone</td>
<td>trace</td>
<td>trace</td>
<td>~100</td>
</tr>
</tbody>
</table>

Methods for hydrolysis:

Several methods of hydrolyzing steroid esters and glycosides are commonly used.

- For the sulfates of DHEA and androstanolone, solvolysis is suitable; this involves treatment with excess organic solvent (e.g., ethyl acetate, dioxan, or tetrahydrofuran) at a temperature of +38°C for 18–24 h.
- Acid hydrolysis at elevated temperatures is a more general method, but has two disadvantages: it can alter the structure and function of the steroids, and the resinated pigments formed need to be removed, because they are present in the extract.
- The third method, enzymatic hydrolysis (with β-glucuronidase and sulfatase), does not involve these drawbacks.

Procedure for the hydrolysis of glucuronides and sulfates in urine:

Protocol:

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adjust the pH of a portion of the sample (10 ml) to 5.5 by adding dilute acetic acid.</td>
</tr>
<tr>
<td>2</td>
<td>Add 1 ml acetic buffer (1 M pH 5.5) and 0.2 ml β-glucuronidase/arylsulfatase solution.</td>
</tr>
<tr>
<td>3</td>
<td>Incubate at a temperature of +37°C for 16 h.</td>
</tr>
<tr>
<td>4</td>
<td>Cool and extract with an appropriate solvent (e.g., chloroform or dichloromethane) to isolate the hydrolysis products.</td>
</tr>
</tbody>
</table>

Specific activity of Glucuronidase:

Activity:

Generally, the β-glucuronidase activity of the preparation is not as high with respect to steroid β-glucuronides as those obtained from the hydrolysis of synthetic phenyl β-glucuronides indicate. However, under certain conditions, results obtained with phenolphthalein β-glucuronide may be comparable with those given by steroid glycosides, such as estradiol β-glucuronide. For instance, at +37°C and pH 4.5, a β-glucuronidase/arylsulfatase preparation that promotes the hydrolysis of 300 μmol of phenolphthalein β-glucuronide in 1 h, also promotes the hydrolysis of 441 μmol of estradiol β-glucuronide in 1 h.

Standard unit:

The standard unit of β-glucuronidase activity is the enzyme activity that increases the rate of release of 4-nitrophenol from 4-nitrophenyl β-D-glucosiduronic acid at a temperature of +25°C and pH 4.5 by 1 μM.

Phenolphthalein unit:

The phenolphthalein unit of β-glucuronidase activity is the enzyme activity that increases the rate of release of phenolphthalein from phenolphthalein β-D-glucosiduronic acid at a temperature of +38°C by 1 μM. Approximately 4.5 standard units are equivalent to 2.6 phenolphthalein units.

Fishman unit:

The Fishman unit (9) of β-glucuronidase activity is the enzyme activity that increases the rate of release of phenolphthalein from phenolphthalein β-D-glucosiduronic acid at a temperature of +38°C by 1 μg. Approximately 1 standard unit is equivalent to 22,000 Fishman units (1 phenolphthalein unit is equivalent to 19,000 Fishman units).

Specific activity of Arylsulfatase:

Standard unit:

The standard unit of arylsulfatase activity is the enzyme activity that increases the rate of release of 4-nitrophenol from 4-nitrophenyl sulfate at a temperature of +25°C and pH 6.2 by 1 μM.

Phenolphthalein unit:

The phenolphthalein unit of arylsulfatase activity is the enzyme activity that increases the rate of release of phenolphthalein from phenolphthalein disulfate at a temperature of +38°C and pH 6.2 by 1 μM. Approximately 5.4 standard units are equivalent to 1 phenolphthalein unit.

Roy unit:

The Roy unit of arylsulfatase activity is the enzyme activity that increases the rate of release of 4-nitropyrocatechol from 2-hydroxy-5-nitrophenyl hydrogen sulfate (4-nitropyrocatechol 2-sulfate) at a temperature of +38°C and pH 6.2 by 1 μg (9). Approximately 1 standard unit is equivalent to 57,000 Roy units (1 phenolphthalein unit is equivalent to 308,000 Roy units).

Specific arylsulfatase activity:

At +25°C and pH 6.2, the arylsulfatase activity of 1 ml of the preparation is 14 standard units, equivalent to 2.6 phenolphthalein units or 800,000 Roy units at +38°C.

Changes to previous version:

Editorial changes.

Ordering Information:

Roche Applied Science provides a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com.

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