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Not for use in diagnostic procedures.

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

Version 09
Content version: March 2017

Store at +2 to + 8°C

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.

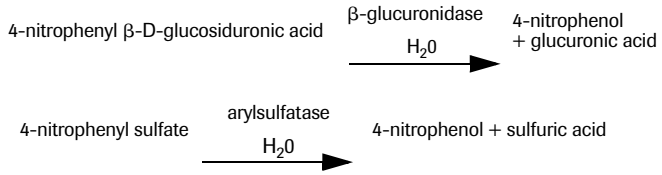
Such compounds include:
- steroids, such as estriol (K_m = 0.42 mM, pH 4.5), androsterone, pregnanediol, tetrahydrocortisone
- phenols, such as phenolphthalein (K_m = 0.39 mM), 4-nitro-phenol, 4-methylumbelliferone
- drugs, such as chloramphenicol and tetrahydrocannabinols
- metabolites, such as thyroxine 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 Acrylsulfatase
Sulfate esters of many phenols are hydrolyzed in the presence of arylsulfatase. Examples are steroid sulfates, such as estronesulfate, 4-nitrophenyl hydrogen sulfate (K_m = 1.8 mM, pH 7.3), 4-nitro-pyrocatechol 2-sulfate (K_m = 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 $mM^{-1} \times l \times cm^{-1}$ 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)

Tab 1: Relative steroid proportions before hydrolysis are given in the following table:

Compound or category	free [%]	sulfate [%]	glucuronide [%]
7-Hydroxycorticosteroids	1	10–15	85–90
Pregnanediol	0	trace	≈ 100
Pregnanetriol	trace	trace	≈ 100
Estrone (O_1)	1–3	10–15	85–89
Estradiols (O_2)	1–3	5–10	90–95
Estriol (O_3)	0–2	5–10	90–95
Androsterone	trace	20	80
Etiocholanolone	trace	10	90
Dehydroepiandrosterone (DHEA)	trace	≈ 100	trace
Epiandrosterone	trace	≈ 100	trace
11 β-Androsterone	trace	10	90
11 β-Etiocholanolone	trace	10	90
11 Ketoandrosteroene	trace	trace	≈ 100
11 Ketoetiocholanolone	trace	trace	≈ 100

Methods for hydrolysis

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

- For the sulfates of DHEA and androsterone, 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 resinified 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

Please refer to the following table.

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

Specific activity of Glucuronidase

Activity

Generally, the β-glucuronidase activity of the preparation is not as high with respect to steroid β-glucuronides, as values 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 5.5 phenolphthalein units.

Fishman unit

The Fishman unit (8) 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 β-glucuronidase activity

At +25°C and pH 4.5, the β-glucuronidase activity of 1 ml of the preparation is 4.5 standard units, equivalent to 5.5 phenolphthalein units or 100,000 Fishman units at +38°C.

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

References

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