For life science research only.
Not for use in diagnostic procedures.

β-Glucuronidase/Arylsulfatase
from *Helix pomatia*

Version: 10
Content Version: June 2021

β-D-Glucuronoside glucuronosohydrolase, Arylsulfate sulfohydrolase

Cat. No. 10 127 060 001	2 ml *Not available in US*
Cat. No. 10 127 698 001	10 ml

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	β-Glucuronidase/ Arylsulfatase	Enzyme mix of β-Glucuronidase/ Arylsulfatase in saline, stabilized with 0.02% sodium azide.	10 127 060 001	1 vial, 2 ml
			10 127 698 001	1 vial, 10 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	β-Glucuronidase/Arylsulfatase	Store at +2 to +8°C. ⚠ ***Alternatively, aliquot and store at −15 to −25°C.*** ⚠ ***Avoid repeated freezing and thawing (more than 2 times).***

1.3. Additional Equipment and Reagent required

For hydrolysis of glucuronides and sulfates in urine

- Acetic acid
- Acetate buffer, 1 M, pH 5.5
- Chloroform or dichloromethane

1.4. Application

β-Glucuronidase/Arylsulfatase exhibits strong enzyme activity and is widely used for the simultaneous hydrolysis of β-glucuronides (β-glucosiduronic acids) and sulfate esters in urine and other biological fluids.

- Enzymatic hydrolysis of steroid β-glucuronides and sulfates.
- Removal of cell walls from yeasts in the preparation of protoplasts in cell biology.
- Enzyme immobilization studies.
- Determination of drugs in urine.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Steroids in urine

The various steroids found in urine may be present in one or more of three forms:
- Free compound, in minor or trace quantities and amounts.
- Sulfate, predominant in some cases.
- β-glucuronide, the predominant form in most cases.

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, such as ethyl acetate, dioxan, or tetrahydrofuran at a temperature of +38°C for 18 to 24 hours.
- 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.

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

Compound/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

Working Solution

In many applications, the product can be diluted with water immediately before use or used undiluted.

The β-Glucuronidase/Arylsulfatase preparation is very concentrated and must be diluted for some applications. In the preparation of protoplasts, the precise concentration to use for a given strain of yeast must be determined empirically.

2.2. Protocols

Hydrolysis of glucuronides and sulfates in urine

1. Adjust the pH of a portion of the sample (10 ml) to 5.5 by adding diluted acetic acid.
2. Add 1 ml acetate buffer (1 M, pH 5.5) and 0.2 ml β-Glucuronidase/Arylsulfatase solution.
3. Incubate at +37°C for 16 hours.
4. Cool and extract with an appropriate solvent to isolate the hydrolysis products, such as chloroform or dichloromethane.

2.3. Parameters

EC-Number

β-D-Glucuronoside glucuronosohydrolase: EC 3.2.1.31
Arylsulfate sulfohydrolase: EC 3.1.6.1

Inhibition

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.

pH Optimum

- β-Glucuronidase activity: pH 4.5 to 5.0
- Arylsulfatase activity: pH 6.2

i *May be greater for some substrates in comparatively high concentrations. For example, for 16.5 mM solutions of 2-hydroxy-5-nitrophenyl hydrogen sulfate, it is pH 7.2.*

Specific Activity

β-Glucuronidase

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.

Arylsulfatase

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.

Specific activity of β-Glucuronidase

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 example, 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 hour, also promotes the hydrolysis of 441 µmol of estradiol β-glucuronide in 1 hour.

Specificity

Specificity of β-Glucuronidase

The glycosides that β-D-glucuronic acid forms with a variety of compounds containing hydroxyl groups hydrolyze readily in the presence of β-Glucuronidase.
Such compounds include:
- Steroids, such as estriol (K_m = 0.42 mM, pH 4.5), androsterone, pregnanediol, and tetrahydrocortisone.
- Phenols, such as phenolphthalein (K_m = 0.39 mM), 4-nitrophenol, and 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 part: neither α-glucosides nor β-glucosiduronic acids are hydrolyzed. However, the nature of the residue linked to the β-glucuronic acid residue is hardly important.

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 (K_m = 1.8 mM, pH 7.3), 4-nitro-pyrocatechol 2-sulfate (K_m = 1.25 mM, pH 7.5), and phenolphthalein disulfate.

Unit Definition

Specific activity of β-Glucuronidase

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 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.

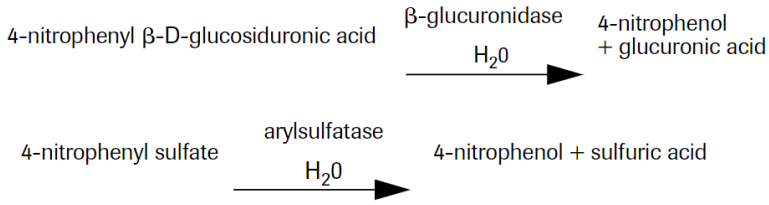
Roy unit

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.
Approximately 1 standard unit is equivalent to 57,000 Roy units (1 phenolphthalein unit is equivalent to 308,000 Roy units).

3. Additional Information on this Product

3.1. Test Principle

Reaction mechanism



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.

3.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Trademarks

All product names and trademarks are the property of their respective owners.

4.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.7. Contact and Support

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

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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