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

β-Glucuronidase
from *E. coli* K 12
β-D-Glucuronoside glucuronosohydrolase

Version: 08
Content Version: April 2021

Cat. No. 03 707 580 001	1 ml *Not available in US*
Cat. No. 03 707 598 001	5 ml
Cat. No. 03 707 601 001	15 ml

Store the product at +2 to +8°C.

1. General Information 3
1.1. Contents 3
1.2. Storage and Stability 3
Storage Conditions (Product) 3
1.3. Application 3
2. How to Use this Product 4
2.1. Before you Begin 4
General Considerations 4
Methods for hydrolysis 4
Steroids in urine 4
2.2. Protocols 5
Method of determination 5
2.3. Parameters 6
EC-Number 6
pH Optimum 6
Specific Activity 6
Specificity 6
Temperature Optimum 6
Unit Definition 6
3. Additional Information on this Product 7
3.1. Test Principle 7
Principle of the standard test 7
4. Supplementary Information 8
4.1. Conventions 8
4.2. Changes to previous version 8
4.3. Trademarks 9
4.4. License Disclaimer 9
4.5. Regulatory Disclaimer 9
4.6. Safety Data Sheet 9
4.7. Contact and Support 9

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	β-Glucuronidase from *E. coli* K 12	Enzyme in 50% glycerol, pH approximately 6.5.	03 707 580 001	1 bottle, 1 ml
			03 707 598 001	1 bottle, 5 ml
			03 707 601 001	1 bottle, 15 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	Store at +2 to +8°C. ⚠ ***Storage at −15 to −25°C may prolong the life of the preparation, but this has not been tested. During the first 6 months, the loss of activity may reach about 10%.***

1.3. Application

Use β-Glucoronidase for
- Hydrolysis of steroid conjugates (glucuronides) in urine at pH 6.0 to 6.5.
- Drug analysis
- Detection of benzodiazepine in small doses.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Methods for hydrolysis

Several methods are commonly used to hydrolyze steroid esters and glycosides:
- 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.

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.

The relative proportions 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-Ketoandrosterone	trace	trace	100
11-Ketoetiocholanolone	trace	trace	100

2.2. Protocols

Method of determination

Compound or category	Parts in sample	Enzyme solution	Hydrolysis temperature / duration [°C] / [min]	Method of determination
7-Hydroxy-cortico-steroids	5	1 drop	37 / 75 42 / 60 46 / 45	Porter and Silber's method.
Estrogens (total)	5 – 50 (varies with concentration)	1 drop/5 ml		▪ Kober's colorimetric method. ▪ Ittrich's fluorometric method.
Pregnanediol[1]	5 – 50 (varies with concentration)	1 drop/5 ml	37 / 40 42 / 30 46 / 20	▪ Talbot's colorimetric method. ▪ Thin-layer chromatography. ▪ Gas chromatography
Estriol (in pregnancy)	5 – 10	2 drops/5 ml		▪ Kober's colorimetric method. ▪ Ittrich's fluorometric method. ▪ Gas chromatography
Pregnanetriol	50	10 drops	37 / 75 42 / 60 46 / 45	▪ Gas chromatography
Estradiol, estrone, estriol	5 – 50	1 drop/5 ml		▪ Separate compounds by gas chromatography; Bauld's modification of Cohen & Marrian's method if only the estrogen coefficient is needed.
17-Ketosteroids[2]	50	10 drops		▪ Gas chromatography ▪ Chromatography in liquid phase.

[1] For 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 solvolysis (18 hours, +38°C) of the sulfate conjugates.

2.3. Parameters

EC-Number

EC 3.2.1.31

pH Optimum

pH 6.0 to 6.5; activity is reduced above or below this range.

Specific Activity

With 4-nitrophenyl-β-D-glucuronide (4NPG) as substrate, the specific activity of β-Glucuronidase is approximately 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.

Specificity

β-Glucuronidases extracted from bovine liver, *Helix pomatia*, *Patella vulgata*, or *E. coli* have been used extensively in research and analytical laboratories for the enzymatic hydrolysis of steroid β-glucuronides.

- However, the bacterial enzyme was very much more active with respect to hydrolysis of estrogen β-glucuronides than that obtained from the Roman snail, even though the ratio of the activity of their *E. coli* β-glucuronidase to the activity of the present preparation was only 0.2 to 5×10^{-2} .
- Because of the high hydrolytic activity of the β-glucuronidase from *E. coli*, steroid β-glucuronides can be hydrolyzed extremely quickly; the reaction can be finished in 15 to 30 minutes.
- Compared with similar enzymes, *E. coli* β-glucuronidase retains its activity much better as the hydrolysis proceeds, since it is 10 times less sensitive to changes in the concentration of the steroid β-glucuronide.

Temperature Optimum

Remains completely stable for 18 hours; at +48°C, it loses 35% of its original activity within 2 hours.

Unit Definition

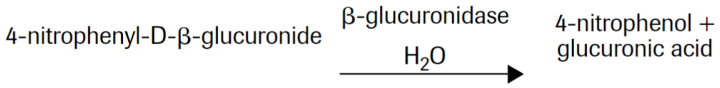
The international unit of β-glucuronidase activity is the enzyme activity that increases the rate of release of 4-nitrophenol from 4-nitrophenyl-β-D-glucuronide (4NPG) at a temperature of +25°C and pH 7.0 by 1 µM.
The Fishman unit was used formerly. This is defined in terms of the release of phenolphthalein 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 catalyze 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:

- Although the Michaelis concentrations for the two substrates are not very dissimilar ($K_M = 2 \times 10^{-4}$ M for 4 NPG and $K_M = 6 \times 10^{-5}$ M for PPG), the corresponding rates of hydrolysis differ more: 4NPG is hydrolyzed about 5 times as fast as PPG.
- In the case of PPG, inhibition through excess substrate is observed; this does not occur with 4NPG.

3. Additional Information on this Product

3.1. Test Principle

Principle of the standard test



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.

0421.03731871001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany