β-Glucuronidase

from *E. coli* K 12
β-D-Glucuronoside glucuronosohydrolase, *EC 3.2.1.31*

Cat. No. 03 707 580 001 1 ml
Cat. No. 03 707 598 001 5 ml
Cat. No. 03 707 601 001 15 ml

Version 07
Content Version: April 2016

Store the kit at −15 to −25°C

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)

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

[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.

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%.

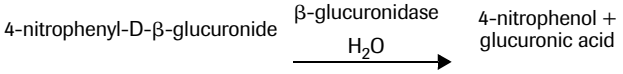
Product description

Specificity of β-Glucuronidase 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, Jayle *et al.* showed that the bacterial enzyme was very much more active with respect to hydrolysis of estrogen β-glucuronides than that obtained from the Roman snail (2), even though the ratio of the activity of their *E. coli* β-glucuronidase to the activity of the present preparation was only $0.2 - 5 \times 10^{-2}$.

Because of the high hydrolytic activity of the β-glucuronidase from *E. coli*, steroid β-glucuronides can be hydrolysed extremely quickly; the reaction can be complete in 15–30 min. Compared with similar enzymes, *E. coli* β-glucuronidase retains its activity much better as the hydrolysis proceeds, since it is 10 × less sensitive to changes in the concentration of the steroid β-glucuronide.

Principle of the standard test



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 ß-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

Methods for hydrolysis

Several methods of hydrolyzing steroid esters and glucuronides are in common use.

- 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 grave disadvantages: it may alter the structures or constitutions of the steroids, and the resinified pigments formed need to be removed specially, since they are taken up in the extract.
- The third method, enzymatic hydrolysis (with β-glucuronidase and sulfatase), does not involve these drawbacks (1)

Advantages

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

Benefit	Feature
High specific activity	Quantitative hydrolysis of steroid compounds in very short time (only 15–50 min, depending on enzyme concentration).
Great affinity for various β-glucuronides	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.
No need for cleaning-up procedures after hydrolysis	During the course of hydrolysis of steroid β-glucuronides, it releases very little of the non-specific chromogens occurring in urine.
No need for buffering urine	Provided the pH value is adjusted to 6.0–6.5, since β-glucuronidase derived from *E. coli* is relatively indifferent to the ionic milieu.

Specific activity of Glucuronidase

Definition of the international β-glucuronidase unit

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 (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^{-4} M for 4 NPG and KM = 6×10^{-5} 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

1 Bradlow, H.L. (1970) "Chemical and Biological Aspects of Steroid Conjugation (Bernstein, S. & Solomon, S., ed.), pp 131, Berlin, Heidelberg, New York.
2 Jayle, M.F., Scholler, R., Jarrige, P. & Mtay, S. (1959) *Bull. Soc. Chim. Biol.* **41**, 1593.
3 Bartsch, W. & Voigt, K.-D. (1985) "Hydrolysis of Steroid Conjugates" in Methods of Enzymatic Analysis 3. edition, Vol. VIII, pp 161, Verlag Chemie, Weinheim.
4 Beck, O. et al. (1992) *Clin. Chem.* **38**, 271-275.
5 Wakabayashi, M. & Fishman, W.H. (1961) *J. Biol. Chem.* **236**, 996.
6 Catlin, D.H. et al. (2002) *Rapid Commun. in Mass Spectrom.* **16**: 1273-1275
7 Breidbach A. & Catlin, D.H. (2001) *Rapid Commun. in Mass Spectrom.* **15**: 2379-2382.
8 Aguilera, R. et al. (1999) *J. Chromatogr. B*, **727**, 95-105.
9 Smith, RF et al. (1996) *Analyst.* 121(1), 67-9.
10 Kourounakis, PN & Tani, E. (1995) *Res . Commun. Mol. Pathol. Pharmacol.* **88(1)**, 119-22.

Ordering Information

Product	Pack Size	Cat. No.
β-Glucuronidase/ Arylsulfatase	2 ml	10 127 060 001

Changes to previous version

Editorial changes

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