Product Information

# MS β-glucuronidase

Pre-buffered, automation-ready hydrolysis mix, 6-12 Reactions/mL

**SRE0103**

EC Number: 3.2.1.31

## Product Description

Most drugs, hormones and xenobiotics are metabolized in humans to hydrophilic molecules and then quickly excreted in body fluids, mainly urine. One of the most common modifications is glucuronidation, which forms glucuronide conjugates. In humans, glucuronidation is the main phase II modification pathway.[1] Figure 1 shows the metabolic step.

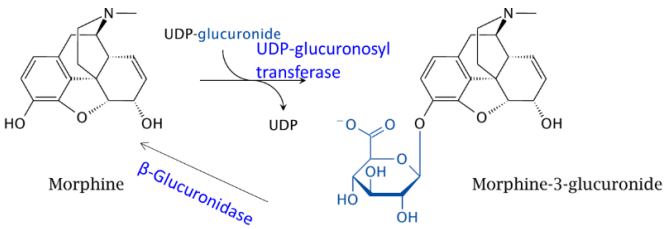


*Figure 1: Depiction of metabolic pathway for glucuronidation of morphine in the liver, and the enzymatic glucuronide hydrolysis that that is used for urine sample preparation.*

Conjugates found in urine are difficult to detect and quantitate by GC, HPLC, and LC/MS. These conjugates are thus commonly cleaved from the drug through hydrolysis with β-glucuronidase (beta-gluc), to form the original drug, hormone or xenobiotic. Released analytes can be screened and quantified with common instrumentation.

MS beta-glucuronidase is specifically designed for medium to high-throughput, automated urine sample testing in toxicology laboratory settings. This is achieved by a mass spectrometry (MS)-compatible, pre-buffered, room temperature (RT)-stable product that allows for a quick and simple walk-away workflow.

## Storage/Stability

Store at 2-8 °C for long-term storage, or for 2 months if stored at -20 °C. The enzyme also retains activity when stored in the Master Mix under ambient conditions for ≥ 7 days.

## Procedure

The Quick Start Guide (QSG) provided with the product provides a simple protocol that can be followed to achieve >80% recoveries for a wide range of analytes, including analytes that are difficult to hydrolyze, such as Codeine. This product and protocol were co-developed using human urine matrix, to ensure that it overcomes the inhibitory characteristics seen in human urine. Additional steps such as SPE or filtration can be added as needed. The product can be adapted to a laboratory's specific SOPs and workflows.

For routine testing, prepare a Master Mix† as in Figure 2 and store for use as needed.

| Master Mix | 20°C | 40°C |
|---|---|---|
| Component (mL) | Vol (mL)* | Vol (mL)* |
| MS β-Gluc (mL) | 16 | 8 |
| DI water (mL) | 0 | 8 |
| ISDs in 100% MetOH (mL) | 2 | 2 |
| Total Volume (mL) | 18 | 18 |

*Figure 2: Preparation instructions for a Master Mix. All volumes can be scaled linearly to fit your needs.*

Follow the workflow provided in Figure 3 to prepare urine samples for analysis. The enzyme is stable in the Master Mix up to 7 days at room temperature. The stability of internal standards should be checked on a case-by-case basis. Allow the working Master Mix solution to reach room temperature (≥ 20 °C) before use.

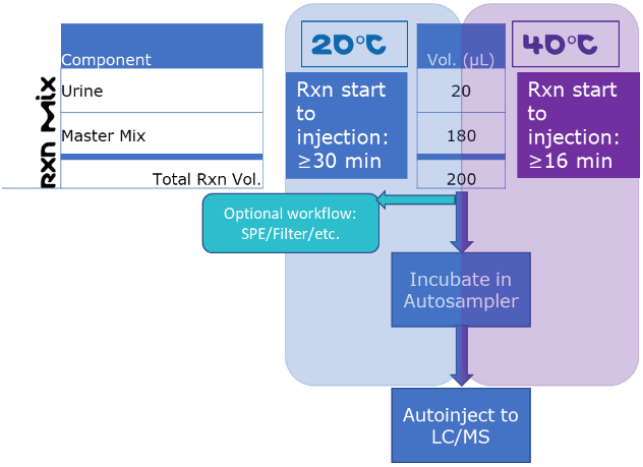


*Figure 3: Quick Start Workflow. All volumes can be scaled linearly to fit your needs. This is designed with the Dilute and Shoot workflow in mind, but can be adapted for SPE, filtration, etc. as needed.*

## Routine sample Stepwise Protocol

1. Set the autosampler to your desired temperature.
2. Pipette urine into reaction vessel (vial, plate or compatible SPE configuration).
3. Add Master Mix.
4. Transfer to the autosampler.ǂ
5. After 16 minutes at 40 °C, or 30 minutes at 20 °C, inject the hydrolyzed specimens.

Single samples can be hydrolyzed for analysis without the need for preparing a Master Mix by adding the components in Table 1.

| Component | 20°C | 40°C |
|---|---|---|
| Urine (µL) | 20 | 20 |
| DI-Water (µL) | 0 | 80 |
| Enzyme (µL) | 160 | 80 |
| ISDs in 100% MetOH (µL) | 20 | 20 |
| Total Vol (µL) | 200 | 200 |

*Table 1: Reaction mixture for single sample preparation (no Master Mix needed).*

## Results

Drug recovery values were obtained through LC/MS with glucuronide-conjugated and free analytes as standards for quantitation. Table 2 shows recoveries achieved under the conditions recommended by the above procedure.

| Analyte | 20°C, 30min | 40°C, 16min |
|---|---|---|
| Codeine | >80% | >82% |
| Morphine | >85% | >95% |
| Oxymorphone | >95% | 100% |

*Table 2: Recoveries seen by LC/MS for common analytes when following the Quick Start Guide.*

Analytes are hydrolyzed at different rates, so it is possible for the reaction to complete much faster than indicated, depending on the analyte mix. Oxymorphone, for example, can achieve >80% recovery in approximately 5 minutes at 40 °C. A time course for recovery of 3 analytes (Codeine, Morphine, and Oxymorphone) is shown in Figure 4.



 

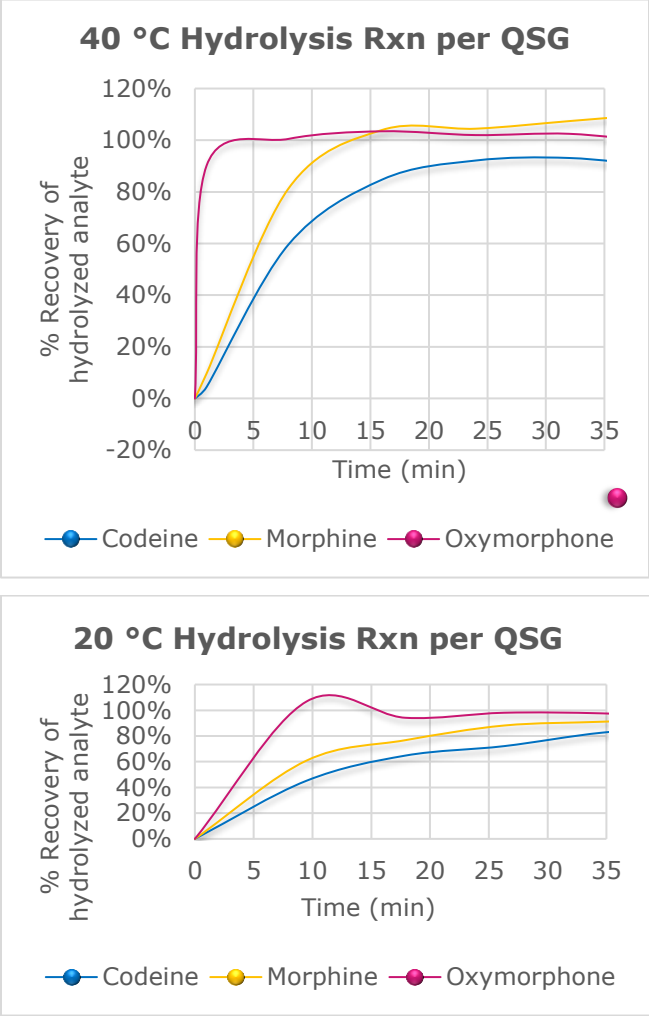


*Figure 4: Time course showing analyte recoveries for a range of drug-glucuronides in human urine under the 40 °C (top panel) and 20 °C (bottom panel) workflows recommended.*

## Notes

* The protocol can be applied to any urine volume by maintaining the given proportions.

† Master Mix is not necessary for individual samples, for occasional testing or method development. To prepare samples without Master Mix, create a reaction mix as in Table 1.

‡ If following SPE, Filtration, or any protocol besides Dilute and Shoot, incubate the reaction before continuing.

## References

1. Cretol, S. *et al*., Pharmacogenetics of phase I and phase II drug metabolism. *Curr. Pharm. Des.*, **16(2),** 204-219 (2009).

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