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β -GLUCURONIDASE REAGENT PACK

PRODUCT #301605

FORENSIC USE ONLY

INTENDED USE: For use with Neogen's Benzodiazepine Group (Oxazepam/Clonazepam) Kit #130119 and #130115

DESCRIPTION

The procedure outlined below will hydrolyze the glucuronic acid from Lorazepam Glucuronide to yield unconjugated Lorazepam for better detection of total Lorazepam in Neogen's Benzodiazepine Group (Oxazepam/Clonazepam) Kit.

The β -Glucuronidase sample treatment for Lorazepam Glucuronide should be able to be utilized for other glucuronide metabolites of other drugs. The β -Glucuronidase sample treatment outlined in this protocol has only been tested on Lorazepam Glucuronide and Neogen's Benzodiazepine Group (Oxazepam/Clonazepam) Kit. It has NOT been tested on any other glucuronide metabolites and/or forensic detection kits. Please contact your Neogen representative for further assistance.

MATERIALS PROVIDED

1. **Reagent A:** 115 mL
2. **Reagent B:** 3 mL

MATERIALS NEEDED BUT NOT PROVIDED

1. β -Glucuronidase lyophilized recombinant from *E.coli* (Sigma Aldrich, Product # G8420-25KU)
2. 37°C Incubator
3. 12 x 75 mm glass culture tubes
4. Culture tube caps or plastic film for covering culture tubes
5. Precision pipettes with a range of 10 μ L - 1,000 μ L
6. Disposable pipette tips
7. Vortex

STORAGE AND STABILITY

Reagent B must be stored at -20°C. **Reagent A** must be stored at 4°C. All components of the reagent pack can be used until the expiration date found on the label when stored properly.

PRECAUTIONS AND NOTES

1. **DO NOT** use components of reagent pack after their expiration date.
2. **DO NOT** mix components of reagent pack with different reagent pack lots.
3. **DO NOT** pipette reagents by mouth.
4. All specimens should be considered potentially infectious. Exercise proper handling precautions.
5. Use aseptic techniques when opening and removing reagents from containers.
6. **DO NOT** smoke, eat, or drink in areas where specimens and/or reagents are being handled.
7. **DO NOT** incubate β -Glucuronidase treated sample for longer than specified in the procedure as a longer incubation time could affect the hydrolysis process.

PROCEDURE

1. Reconstitute the β -Glucuronidase lyophilized recombinant using **Reagent B**. Sigma Aldrich Product # G8420-25KU contains 2 mg of approximately 12,500 units/mg solid. Add 2 mL of **Reagent B** to yield approximately 12,500 units/mL (U/mL) enzyme concentration.
2. Prepare the β -Glucuronidase solution concentration by diluting the reconstituted β -Glucuronidase in **Reagent A** to 100 U/mL. For example, to prepare 5 mL of a 100 U/mL concentration, add 40 μ L of the reconstituted β -Glucuronidase to 4,960 μ L of **Reagent A**.
3. Add 500 μ L of the 100 U/mL β -Glucuronidase solution to 500 μ L of the sample.
4. Cover the top of the test tube with plastic film or a culture tube cap and briefly vortex for 5 seconds.
5. Place the covered culture tube into a 37°C incubator for 15 minutes.
6. After the 15 minute incubation, remove the tube from the incubator.
7. Follow the Test Procedure from the Benzodiazepine Group (Oxazepam/Clonazepam) kit insert.

NOTE: The sample is diluted 1:2 in the glucuronidase procedure. Take this dilution in consideration when performing your typical sample dilution before assaying. For example, if samples are typically diluted 1:10 before assaying, dilute 1:5 after the glucuronidase procedure to give a final dilution of 1:10.

RECOVERY BEFORE AND AFTER β -GLUCURONIDASE TREATMENT

%Recovery of Lorazepam from Lorazepam Glucuronide
Before and After Beta-Glucuronidase Treatment

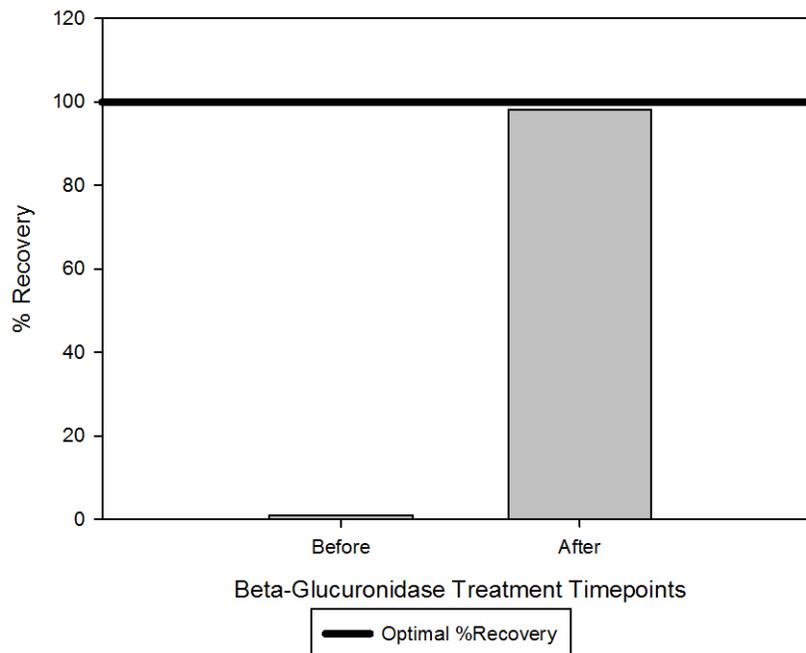


Table 1. Concentration of β -Glucuronidase in human urine before and after treatment.

Human Urine Sample	Concentration <i>before</i> β -Glucuronidase Treatment (ng/mL)	Concentration <i>after</i> β -Glucuronidase Treatment (ng/mL)
Sample 1	97.3	355.1
Sample 2	11.7	3393

TECHNICAL SUPPORT

For technical assistance, please contact our technical services department at (859) 254-1221 or email at techservice-toxicology@neogen.com. Representatives are available Monday – Friday from 8:00 am – 6:00 pm EST.

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