

# APPLICATIONS

## A Quick $\beta$ -Glucuronidase Enzyme Removal Procedure Using $\beta$ -Gone<sup>TM</sup> $\beta$ -Glucuronidase Removal for the Analysis of Naltrexone

Matthew Brusius  
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

### Introduction

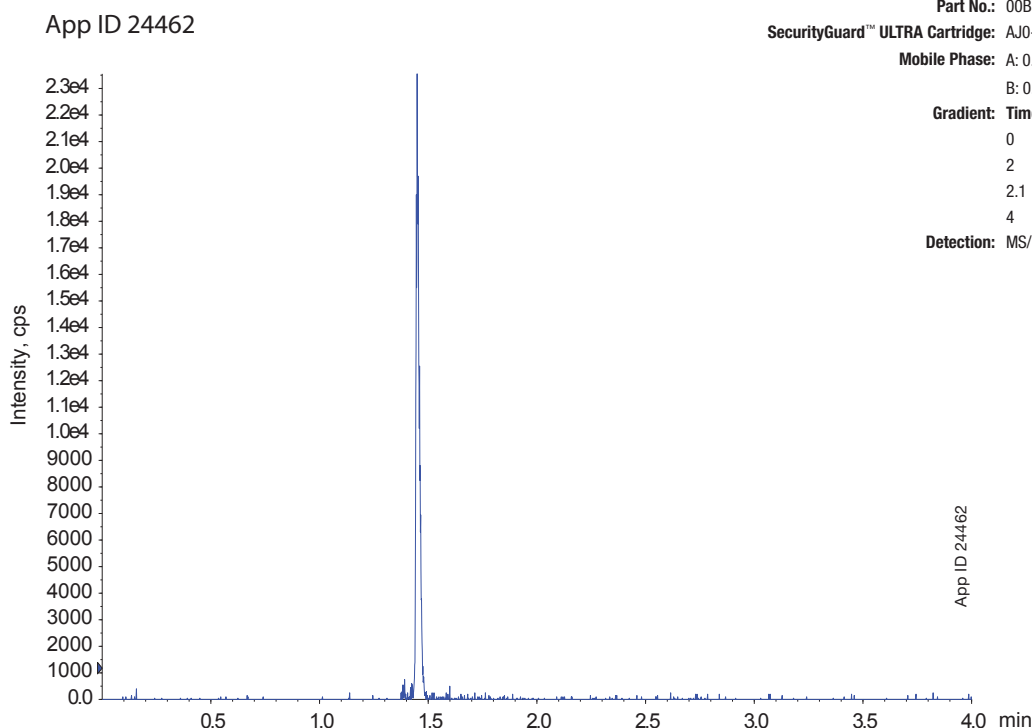
This application describes how  $\beta$ -Gone  $\beta$ -Glucuronidase Removal Products can be used for the analysis of naltrexone from urine that has been hydrolyzed using the  $\beta$ -Glucuronidase Enzyme BG100<sup>TM</sup> (Kura Biotec SpA). By selectively removing the  $\beta$ -glucuronidase, column lifetime is improved versus a standard “dilute-and-shoot” protocol while achieving exceptional recovery for the analyte of interest.

### Materials and Methods

All reagents and solvents were HPLC or analytical grade. Analyses were performed using an API 4000<sup>TM</sup> LC-MS/MS (SCIEX, Framingham, MA).

Average % Recovery	% CV (n=5)
100	3

Representative XIC for Naltrexone in Urine After Cleanup with  $\beta$ -Gone



### Sample Preparation

#### Enzymatic Hydrolysis

BG100 Enzyme Hydrolysate was prepared as follows:

- 1) Add 10  $\mu$ L of analyte spike (1  $\mu$ g/mg) to 200  $\mu$ L urine
- 2) Dilute with 100  $\mu$ L of 0.1 M ammonium acetate buffer and add 40  $\mu$ L of Kura BG100 Beta Glucuronidase Solution from Red Abalone, Activity >100KU/mL (Kura Biotec, Part No.: 9001-45-0, Lot No. 4022)
- 3) Add 400  $\mu$ L 0.1 % Formic acid

#### $\beta$ -Gone Protocol

- 1) Dilute 200  $\mu$ L of Urine Hydrolysate with 133  $\mu$ L of 0.1 % Formic acid in Methanol
- 2) Load diluted sample onto  $\beta$ -Gone 96-Well Plate (Part No.: 8E-S322-DGA) and apply 2-5 psi using a positive pressure manifold or a vacuum manifold
- 3) Collect eluent and inject 5  $\mu$ L for analysis

### HPLC Conditions

**Columns:** Kinetex<sup>®</sup> 2.6  $\mu$ m Biphenyl 100Å  
**Dimensions:** 50 x 2.1 mm  
**Part No.:** 00B-4622-AN  
**SecurityGuard<sup>TM</sup> ULTRA Cartridge:** AJ0-8781  
**Mobile Phase:** A: 0.1 % Formic acid in Water  
 B: 0.1 % Formic acid in Acetonitrile  
**Gradient:**

Time (min)	% B
0	5
2	100
2.1	5
4	5

**Detection:** MS/MS (SCIEX API 4000)



# APPLICATIONS

## Ordering Information

### β-Gone™ β-Glucuronidase Removal Products

Part No.	Description	Unit
8B-S139-TAK	1 mL Tubes, Recombinant Enzyme	100/Box
8B-S322-DAK	1 mL Tubes, Non-Recombinant Enzyme	100/Box
8E-S139-TGA	96-Well Plate, Recombinant Enzyme	1/Box
8E-S322-DGA	96-Well Plate, Non-Recombinant Enzyme	1/Box
8N-S323-TUK	2 mL Centrifuge Tubes, Recombinant and Non-Recombinant Enzyme	100/Box



### Kinetex<sup>®</sup> 2.6 μm Minibore Columns (mm)

Phases	SecurityGuard™ ULTRA Cartridges <sup>†</sup>				3/pk
	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	
Biphenyl	00A-4622-AN	00B-4622-AN	00D-4622-AN	00F-4622-AN	AJO-9209

for 2.1 mm ID



If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

### Kinetex 2.6 μm MidBore™ Columns (mm)

Phases	SecurityGuard™ ULTRA Cartridges <sup>†</sup>			3/pk
	50 x 3.0	100 x 3.0	150 x 3.0	
Biphenyl	00B-4622-YO	00D-4622-YO	00F-4622-YO	AJO-9208

for 3.0 mm ID

<sup>†</sup> SecurityGuard ULTRA Cartridges required holder, Part No.: AJO-9000.

### Presston™ 100 Positive Pressure Manifold

Part No.	Description	Unit
AH0-9334	Presston 100 Positive Pressure Manifold, 96-Well Plate	ea
AH0-9342	Presston 100 Positive Pressure Manifold, 1 mL Tube Complete Assembly	ea
AH0-9347	Presston 100 Positive Pressure Manifold, 3 mL Tube Complete Assembly	ea
AH0-9343	Presston 100 Positive Pressure Manifold, 6 mL Tube Complete Assembly	ea



**WARRANTY** Phenomenex warrants that for a period of 12 months following delivery, the Presston 100 Positive Pressure Manifold you have purchased will perform in accordance with the published specifications and will be free from defects in materials or workmanship. In the event that the Presston 100 Positive Pressure Manifold does not meet this warranty, Phenomenex will repair or replace defective parts. Please visit [www.phenomenex.com/Presston](http://www.phenomenex.com/Presston) for complete warranty information.

#### Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions which may be viewed at [www.phenomenex.com/TermsAndConditions](http://www.phenomenex.com/TermsAndConditions).

#### Trademarks

Kinetex is a registered trademark of Phenomenex. β-Gone, Presston, MidBore, and SecurityGuard are trademarks of Phenomenex. BG100™ is a trademark of Kura Biotec SpA. API 4000™ is a trademark of AB SCIEX Pte. Ltd. AB SCIEX™ is being used under license.

#### Disclaimer

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

Phenomenex is not affiliated with Kura Biotec SpA.

© 2017 Phenomenex, Inc. All rights reserved.

#### Australia

t: +61 (0)2-9428-6444  
f: +61 (0)2-9428-6445  
auiinfo@phenomenex.com

#### Austria

t: +43 (0)1-319-1301  
f: +43 (0)1-319-1300  
anfrage@phenomenex.com

#### Belgium

t: +32 (0)2 503 4015 (French)  
t: +32 (0)2 511 8666 (Dutch)  
f: +31 (0)30-2383749  
beinfo@phenomenex.com

#### Canada

t: +1 (800) 543-3681  
f: +1 (310) 328-7768  
info@phenomenex.com

#### China

t: +86 400-606-8099  
f: +86 (0)22 2532-1033  
phen@agela.com

#### Denmark

t: +45 4824 8048  
f: +45 4810 6265  
nordicinfo@phenomenex.com

#### Finland

t: +358 (0)9 4789 0063  
f: +45 4810 6265  
nordicinfo@phenomenex.com

#### France

t: +33 (0)1 30 09 21 10  
f: +33 (0)1 30 09 21 11  
franceinfo@phenomenex.com

#### Germany

t: +49 (0)6021-58830-0  
f: +49 (0)6021-58830-11  
anfrage@phenomenex.com

#### India

t: +91 (0)40-3012 2400  
f: +91 (0)40-3012 2411  
indiainfo@phenomenex.com

#### Ireland

t: +353 (0)1 247 5405  
f: +44 1625-501796  
eirinfo@phenomenex.com

#### Italy

t: +39 051 6327511  
f: +39 051 6327555  
italiainfo@phenomenex.com

#### Luxembourg

t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
nlinfo@phenomenex.com

#### Mexico

t: 01-800-844-5226  
f: 001-310-328-7768  
tecnicomx@phenomenex.com

#### The Netherlands

t: +31 (0)30-2418700  
f: +31 (0)30-2383749  
nlinfo@phenomenex.com

#### New Zealand

t: +64 (0)9-4780951  
f: +64 (0)9-4780952  
nzinfo@phenomenex.com

#### Norway

t: +47 810 02 005  
f: +45 4810 6265  
nordicinfo@phenomenex.com

#### Puerto Rico

t: +1 (800) 541-HPLC  
f: +1 (310) 328-7768  
info@phenomenex.com

#### Spain

t: +34 91-413-8613  
f: +34 91-413-2290  
espinfo@phenomenex.com

#### Sweden

t: +46 (0)8 611 6950  
f: +45 4810 6265  
nordicinfo@phenomenex.com

#### United Kingdom

t: +44 (0)1625-501367  
f: +44 (0)1625-501796  
ukinfo@phenomenex.com

#### USA

t: +1 (310) 212-0555  
f: +1 (310) 328-7768  
info@phenomenex.com

#### All other countries Corporate Office USA

t: +1 (310) 212-0555  
f: +1 (310) 328-7768  
info@phenomenex.com

## www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at [international@phenomenex.com](mailto:international@phenomenex.com)