FORENSICS

UCT

BETA-GLUCURONIDASE

PRODUCT LINE

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INNOVATION THROUGH CHEMISTRY

Purified Beta-Glucuronidase Formula
Clean, Rapid And Reliable

Shown from left to right: Abalonase™ purified Beta-glucuronidase formula, Selectrazyme® and Red Abalone Beta-Glucuronidase enzyme from an alternate supplier.

Abalonase™

Purified Beta-glucuronidase formula that has been designed to quickly hydrolyze conjugated drug metabolites in human samples within minutes.

Part Number	Vol. (mL)	Activity (units)
ASBETA-GLUC-10	10	≥50,000 units/mL
ASBETA-GLUC-25	25	≥50,000 units/mL
ASBETA-GLUC-50	50	≥50,000 units/mL
ASBETA-GLUC-100	100	≥50,000 units/mL

Form: Clear Aqueous Solution
Sulfatase Activity: None
Storage: +2°C to +8°C
Effective pH: 4.5
Stability: When properly stored, the enzyme will maintain activity for at least 1 Year. After 1 year, it is recommended that the activity level be reassessed.

Abalonase™ +

Designed for deconjugation of both glucuronidated and sulfated metabolites. The formula is enriched with 4 arylsulfatases making it ideal for the hydrolysis of steroid metabolites.

Part Number	Vol. (mL)	Activity (units)
ASFBETA-GLUC-10	10	≥50,000 units/mL
ASFBETA-GLUC-25	25	≥50,000 units/mL
ASFBETA-GLUC-50	50	≥50,000 units/mL
ASFBETA-GLUC-100	100	≥50,000 units/mL

Form: Clear Aqueous Solution
Sulfatase Activity: > 400 U/mL
Storage: +2°C to +8°C
Effective pH: 5.0
Stability: When properly stored, the enzyme will maintain activity for at least 1 year. After 1 year, it is recommended that the activity level be reassessed.

Technology developed by ango

Benefits:

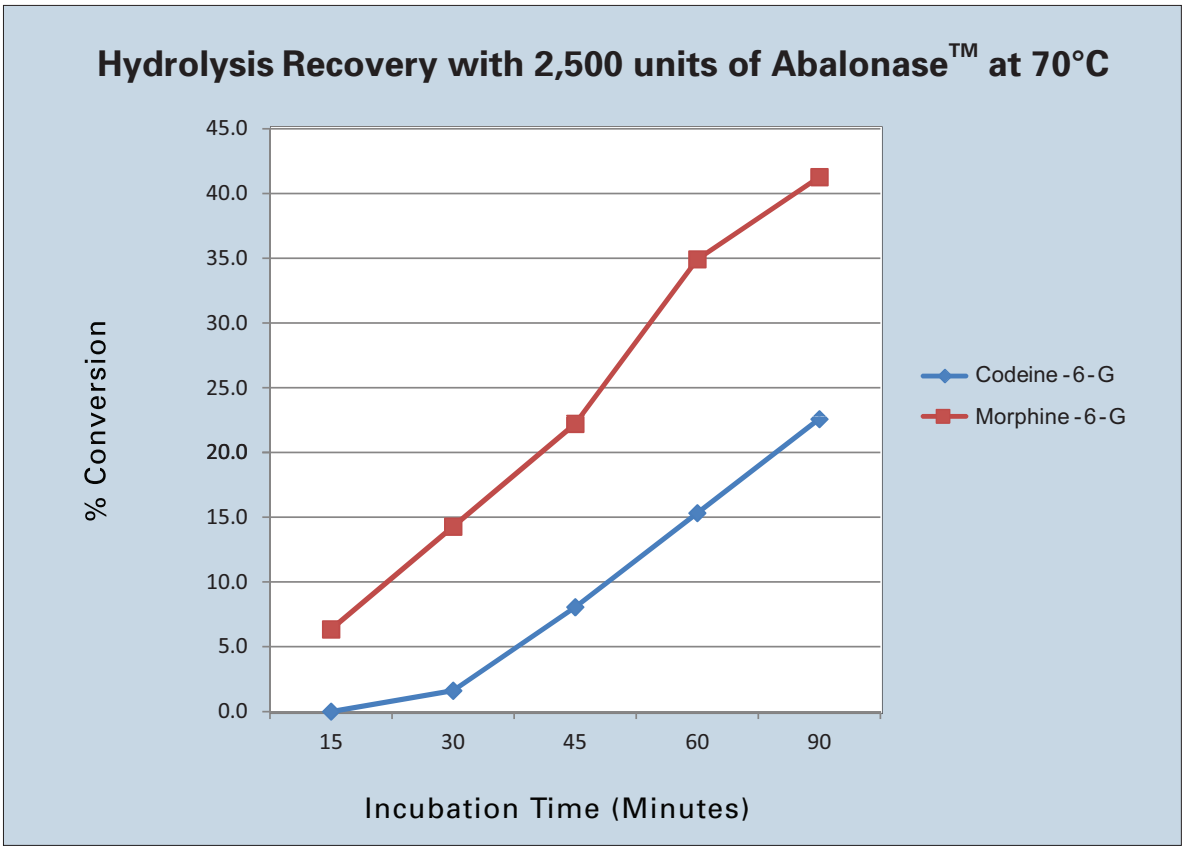
- More efficient than traditional Beta-glucuronidase enzyme sources where half the activity level can achieve comparable or better hydrolysis recoveries.
    - Abalonase™ enzyme activity >50,000 units/mL
    - Selectrazyme® enzyme activity >100,000 units/mL
- Ultra-pure Beta-glucuronidase formula which is designed to not only quickly hydrolyze conjugated drug metabolites in biological samples, but also to obtain cleaner samples, protect analytical columns and minimize laborious equipment maintenance.
- Rapid Hydrolysis Buffer included in every order of either Abalonase™ or Abalonase™ +. Through use of the provided buffer, purified Beta-glucuronidase formulas will achieve their maximum performance. In addition, it will significantly reduce overall sample preparation times.

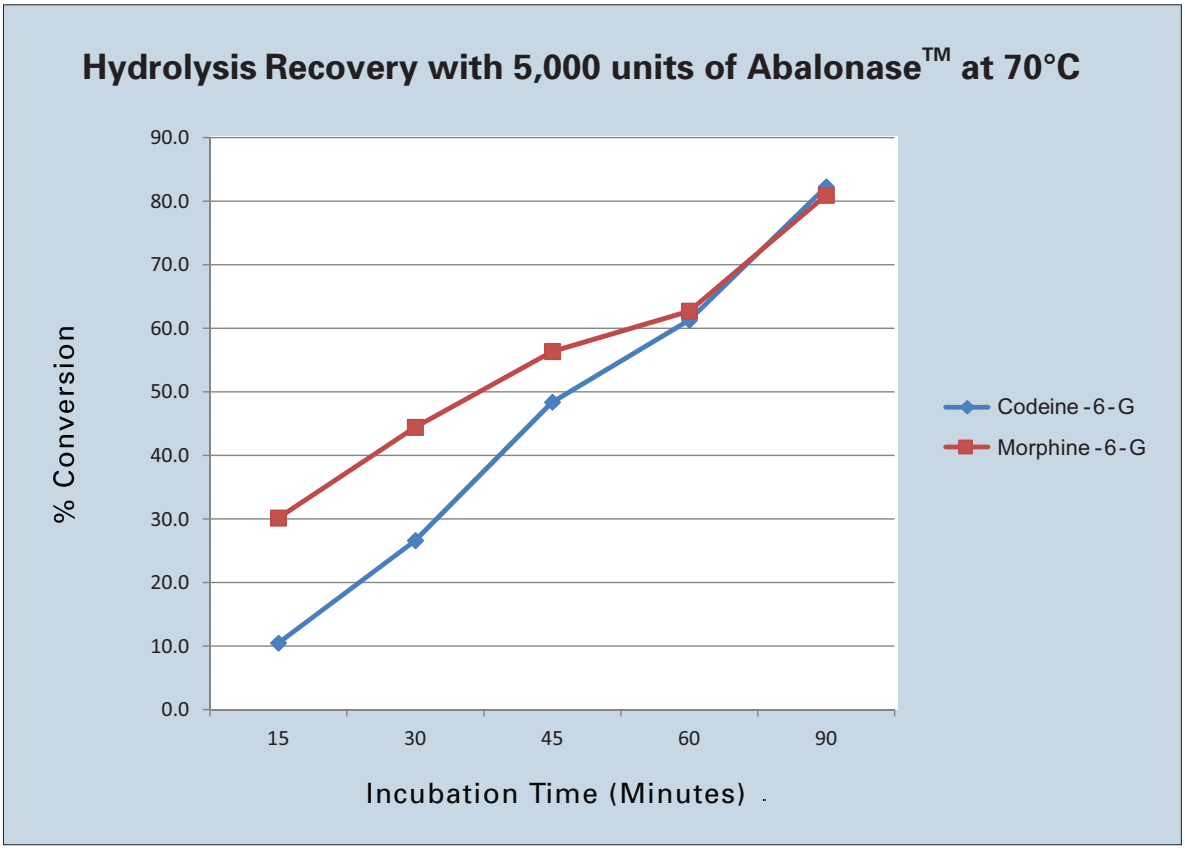
Abalonase™ and Abalonase™+ possess enhanced catalytic efficiency, where half the units of activity provide the same metabolic conversion rate as a traditional-abalone derived enzyme. Acceptable hydrolysis of Morphine-6-Glucuronide and Codeine-6-Glucuronide, crucial urinary metabolites known for their difficulty converting back to free form, was achieved in a reasonable time frame without the addition of exorbitant amounts of enzyme.

Morphine-6-Glucuronide and Codeine-6-Glucuronide Conversion Rates (%)

Enzyme Source	Units of Activity		Morphine-6-G Incubation Time		Codeine-6-G Incubation Time	
			60 min.	90 min.	60 min.	90 min.
Abalonase™	2,500	50 µL	35	41	15	23
	5,000	100 µL	63	81	61	82
Selectrazyme®	5,000	50 µL	27	50	24	36
	10,000	100 µL	44	81	40	65

* All tests run at 70°C, with the included Rapid Hydrolysis Buffer.





* Drug free urine was fortified with both opiate glucuronides at a concentration of 100 ng/mL to liberate 62 ng/ml of each, respectively, upon complete hydrolysis.

HYDROLYSIS PROTOCOL ABALONASE™

Form: Clear liquid solution.
Storage temperature: +2 to +8 °C.

Included Reagents

1) Liquid Abalonase™ / Abalonase™+ (>50,000 units/mL).
2) 10x Rapid Hydrolysis Buffer for Abalonase™ / Abalonase™+.

Working Enzyme Stock Solution Preparation (10,000 Fishman units/mL)

Add respective amount of D.I. H_2O to the 10x Rapid Hydrolysis Buffer. Next, add the stock Abalonase™/ Abalonase™+ to the diluted Rapid Hydrolysis Buffer prepared in the previous step. Refer to table below for corresponding volumes.

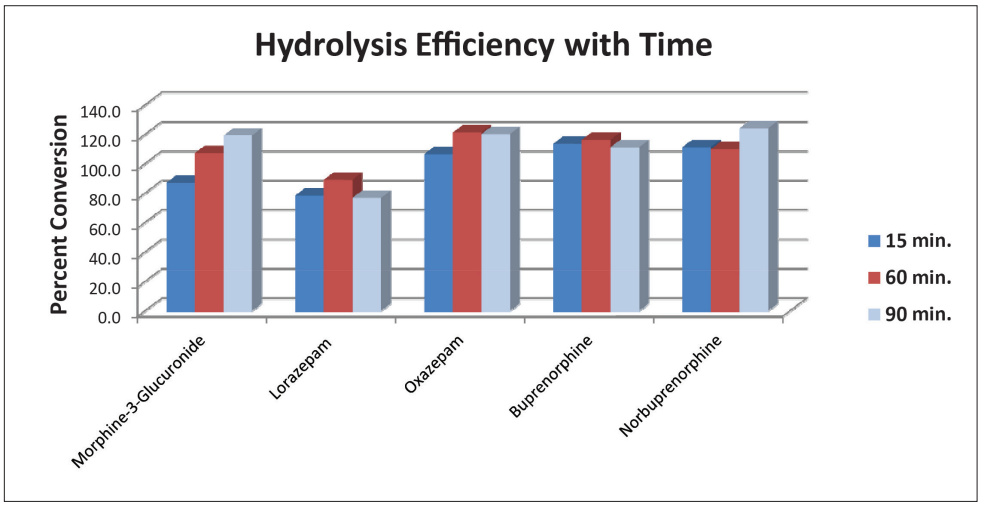
Rapid Hydrolysis Buffer (mL)	D.I. H_2O (mL)	Abalonase ™ / Abalonase ™+ (mL)
4	36	10
10	90	25
20	180	50
40	360	100

*Prepare daily for best results

Urine Sample Hydrolysis

1) To 1 mL of urine sample add 1 mL of Abalonase™/ Abalonase™+ working enzyme stock solution (10,000 Fishman units/mL).
2) Add internal standard(s).
3) Gently mix by inversion or vortex 10 to 15 seconds prior to use.
4) Hydrolyze for 15 minutes to overnight from room temperature to 70°C depending on hydrolysis needs and analytes in question.

*Buffer and stock enzyme can be added separately if desired.



* All tests run at 70°C, with the included Rapid Hydrolysis Buffer.

SELECTRAZYME®

Benefits

- **Available in both liquid and lyophilized forms**
  - Liquid enzyme activity > 100,000 units/mL
  - Lyophilized enzyme activity 1,000,000 - 3,200,000 units/g
- **Abalone derived enzyme**
  - Better hydrolysis efficiency for opiates, benzodiazepines, and steroids than ß-glucuronidase derived from other species
- **Sourced from cultured abalone**
  - Wild abalone populations are not impacted
  - Constant and quick supply of product by using a farm raised source

Liquid:

Part Number	Vol. (mL)	Activity (units)
BETA-GLUC-10	10	≥100,000 units/mL
BETA-GLUC-25	25	≥100,000 units/mL
BETA-GLUC-50	50	≥100,000 units/mL

Storage: +2°C to +8°C
Stability: When properly stored the enzyme will maintain activity for at least 1 year. After 1 year it is recommended that the enzyme activity level be re-tested.

Lyophilized Powder:

Part Number	Activity (units)
BETA-GLUC-250KU	250,000
BETA-GLUC-500KU	500,000
BETA-GLUC-1MU	1,000,000
BETA-GLUC-2MU	2,000,000

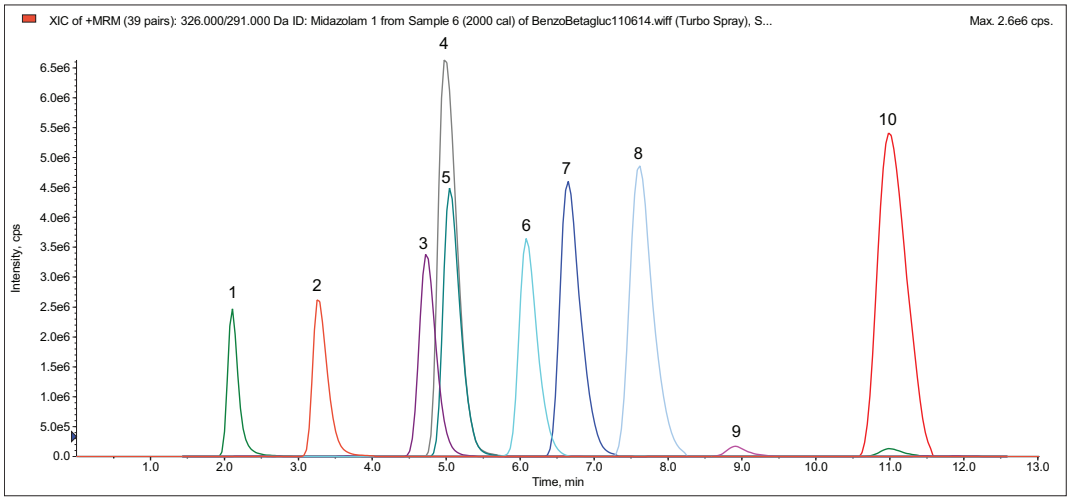
Storage: -20°C
Stability: When properly stored the lyophilized powder will maintain activity for at least 3 years. After 3 years it is recommended that the enzyme activity level be re-tested.

Benzodiazepines Recovery Comparison
Selectrazyme® ß-Glucuronidase Reagent vs. 2 competitors

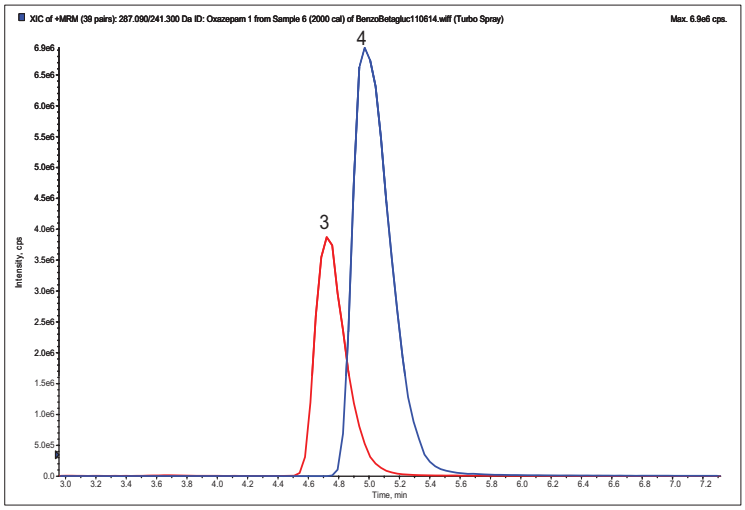
Lorazepam-Glucuronide				
Spiked Concentration	Lorazepam Equivalent	UCT (ng/mL) (n=5)	Vendor 1 (ng/mL) (n=5)	Vendor 2 (ng/mL) (n=5)
100 ng/mL	65 ng/mL	64.5 (99.2%)	64.1 (98.6%)	61.0 (93.8%)
1000 ng/mL	650 ng/mL	690.0 (106%)	687.0 (105%)	684.0 (105%)

Oxazepam-Glucuronide				
Spiked Concentration	Oxazepam Equivalent	UCT (ng/mL) (n=5)	Vendor 1 (ng/mL) (n=5)	Vendor 2 (ng/mL) (n=5)
100 ng/mL	62 ng/mL	71.0 (114%)	69.6 (112%)	67.3 (108%)
1000 ng/mL	620 ng/mL	798.0 (128%)	801.0 (129%)	788.0 (127%)

Benzodiazepine Extracted Standard Chromatogram



Hydrolyzed Lorazepam and Oxazepam Chromatogram



Analyte		MRM Transitions		Relative Retention Time (minutes)
		Q1	Q3	
1.	7-Aminoclonazepam	286.0	222.3	2.10
2.	Midazolam	326.0	291.0	3.26
3.	Lorazepam	321.0	303.3	4.73
4.	Oxazepam	287.0	241.3	4.98
5.	Clonazepam	316.1	270.2	5.05
6.	Alpha-Hydroxy-Alprazolam	325.1	297.1	6.08
7.	Nordiazepam	271.0	140.1	6.65
8.	Temazepm	301.1	255.2	7.59
9.	Alprazolam	309.1	205.3	8.91
10.	Diazepam	285.1	193.2	10.98

Opiates Recovery Comparison
Selectrazyme® ß-Glucuronidase Reagent vs. 2 competitors

Two concentrations of morphine-3-glucuronide, morphine-6-glucuronide, and codeine-6-glucuronide were all individually spiked into blank urine samples in triplicate and analyzed following enzymatic hydrolysis and solid phase extraction. The efficiency of UCT's concentrated Selectrazyme® ß-glucuronidase was compared to two other commercially available abalone derived enzymes. Mean results are included in the tables below.

Morphine-3-Glucuronide				
Spiked Concentration	Morphine Equivalent	UCT (ng/mL) (n=3)	Vendor 1 (ng/mL) (n=3)	Vendor 2 (ng/mL) (n=3)
100 ng/mL	62 ng/mL	59.3 (95.6%)	58.3 (94.0%)	58.06 (93.6%)
1000 ng/mL	620 ng/mL	568.6 (91.7%)	572.0 (92.2%)	579.0 (93.3%)

Morphine-6-Glucuronide				
Spiked Concentration	Morphine Equivalent	UCT (ng/mL) (n=3)	Vendor 1 (ng/mL) (n=3)	Vendor 2 (ng/mL) (n=3)
100 ng/mL	62 ng/mL	28.3 (45.6%)	33.9 (54.7%)	21.3 (34.3%)
1000 ng/mL	620 ng/mL	232.6 (37.5%)	329.6 (53.1%)	217.0 (35.0%)

Codeine-6-Glucuronide				
Spiked Concentration	Codeine Equivalent	UCT (ng/mL) (n=3)	Vendor 1 (ng/mL) (n=3)	Vendor 2 (ng/mL) (n=3)
100 ng/mL	62 ng/mL	12.1 (19.6%)	13.8 (23.9%)	7.04 (11.3%)
1000 ng/mL	620 ng/mL	121.6 (19.6%)	177.6 (28.6%)	111.3 (17.9%)

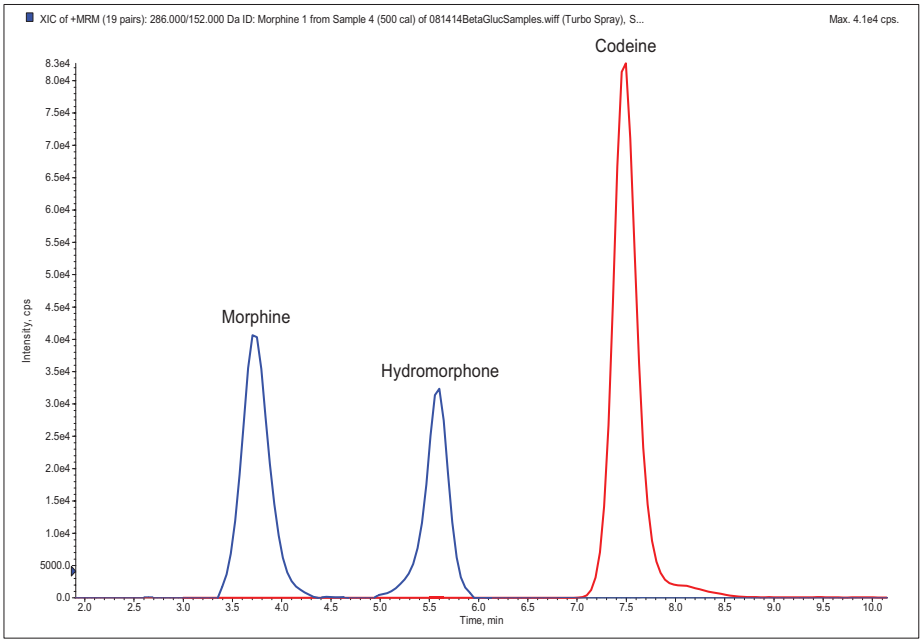
In a second experiment, ten patient urine samples that screened positive for opiates were hydrolyzed using enzyme from UCT and two other commercially available vendors. The mean results are in the tables that follow.

Patient Sample Results

Morphine Concentration (ng/mL)			
Patient Samples	UCT (n=3)	Vendor 1 (n=3)	Vendor 2 (n=3)
1	0	0	0
2	32.6	31.0	30.9
3	197.0	198.0	210.0
4	2645.0	2915.0	2595.0
5	860.5	821.5	811.0
6	0	0	0
7	0	0	0
8	797.0	757.5	659.0
9	0	0	0
10	729.0	753.0	694.0

Codeine Concentration (ng/mL)			
Patient Samples	UCT (n=3)	Vendor 1 (n=3)	Vendor 2 (n=3)
1	0	0	0
2	41.0	49.0	24.7
3	0	0	0
4	0	0	0
5	150.5	142.5	139.0
6	0	0	0
7	0	0	0
8	2710.0	3015.0	2045.0
9	0	0	0
10	0	0	0

Extracted Opiates Standard Chromatogram

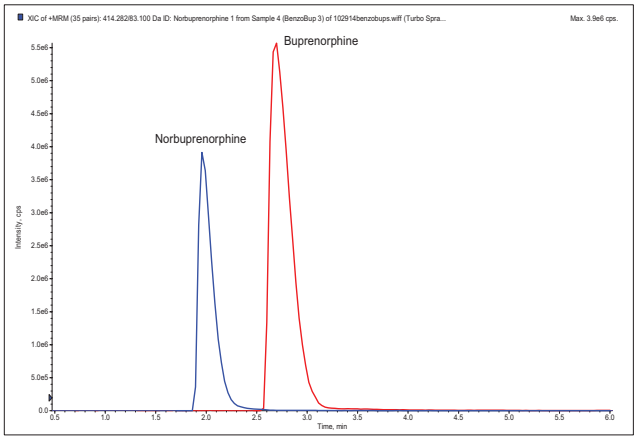


Buprenorphine and Norbuprenorphine Recovery Comparison Selectrazyme® ß-Glucuronidase Reagent vs. 2 competitors

Buprenorphine-Glucuronide				
Spiked Concentration	Buprenorphine Equivalent	UCT (ng/mL) (n=5)	Vendor 1 (ng/mL) (n=5)	Vendor 2 (ng/mL) (n=5)
100 ng/mL	73.0 ng/mL	62.5 (85.6%)	62.3 (85.4%)	62.1 (85.0%)
1000 ng/mL	730.0 ng/mL	778.3 (106%)	773.3 (105%)	768.0 (105%)

Norbuprenorphine-Glucuronide				
Spiked Concentration	Norbuprenorphine Equivalent	UCT (ng/mL) (n=5)	Vendor 1 (ng/mL) (n=5)	Vendor 2 (ng/mL) (n=5)
100 ng/mL	70.0 ng/mL	70.4 (100%)	70.0 (100%)	70.5 (100%)
1000 ng/mL	700.0 ng/mL	824.0 (117%)	826.3 (118%)	835.6 (119%)

Extracted Buprenorphine and Norbuprenorphine Chromatogram



Analyte		MRM Transitions		Relative Retention Time (minutes)
		Q1	Q3	
1.	Norbuprenorphine-D3	417.2	83.1	1.95
2.	Norbuprenorphine	414.2	115.1	1.97
3.	Buprenorphine-D4	472.2	59.2	2.67
4.	Buprenorphine	468.4	55.1	2.69

Conclusion

The results demonstrate that the efficiency of UCT's Selectrazyme® ß-Glucuronidase to cleave the parent drugs from their conjugates compares to other commercially available abalone-derived products. Hydrolysis of commonly encountered opiates, benzodiazepines, buprenorphine and norbuprenorphine all generate comparable results whether using UCT's or competitor enzymes.

Hydrolysis Protocol

1. **REAGENTS**
   Acetic Acid, glacial (CH_3COOH):17.4 M
   Lyophilized ß-Glucuronidase: 1,000,000 – 3,200,000 units/g
   Liquid ß-Glucuronidase: >100,000 units/mL
   Sodium Acetate Trihydrate ($NaCH_3COO\text{-}_3H_2O$): F.W. 136.08

2. **PREPARATION OF BUFFERS:**
   • **1.0 M acetate buffer (pH 5.0 or 4.0):**

   Dissolve 42.9 g of sodium acetate trihydrate in 400 mL D.I. H_2O; Add 10.4 mL of glacial acetic acid. Dilute to 500 mL with D.I. H_2O. Mix.

   For benzodiazepines adjust pH to 5.0 ± 0.1 with 1.0 M sodium acetate or 1.0 M acetic acid.
   For opioids adjust pH to 4.0 ± 0.1 with 1.0 M sodium acetate or 1.0 M acetic acid (*).

   (*) Some authors use pH 5.0 so please check this in your own lab conditions.

   Storage: 25°C in glass or plastic.
   Stability: 6 months; Inspect daily for contamination.

   • **100 mM acetate buffer (pH 5.0 or 4.0):**

   Dilute 40 mL of 1.0 M acetate buffer to 400 mL with D.I. H_2O. Mix.

   Storage: 25°C in glass or plastic.
   Stability: 6 months; Inspect daily for contamination.

3. **WORKING ENZYME STOCK SOLUTION PREPARATION - 5,000 Fishman units/mL:**
   Add 10 mL of the stock liquid ß-Glucuronidase to 200 mL of 100 mM acetate buffer.
   Or
   Dissolve the lyophilized ß-Glucuronidase as shown in Table 1.

   Storage: 5°C in plastic or silanized amber bottle.
   Stability: Several days; Prepare daily for best results.

TABLE 1

Lyophilized β-Glucuronidase Activity	Buffer volume
250 KU	~50 mL
500 KU	~100 mL
1 MU	~200 mL
2 MU	~400 mL

4. **URINE SAMPLE HYDROLISIS**
   To 2 mL of urine add 1 mL of ß-Glucuronidase solution. Add internal standard(s).
   Gently mix by inversion 10 - 15 seconds prior to use. Hydrolyze for 2 - 3 hours at 60 - 65°C.

5. **NOTES**
   Enzyme concentration used can vary from 1,660 to 3,330 units/urine mL, and will affect the hydrolysis time needed. Please be advised that this protocol is just a suggested protocol, therefore it should be confirmed empirically.

6. **ALTERNATE PROCEDURE FOR URINE SAMPLE HYDROLYSIS USING THE ADDITION OF STOCK (≥100,000 Units/mL) ß-GLUCURONIDASE IN THE LIQUID FORM**

   To 1 mL of urine sample add 1 mL 1M acetate buffer solution and 50 µL of stock liquid ß-glucuronidase.

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