

# A summary of “Optimization of Extraction Parameters using IMCSzyme® β-glucuronidase for Blood Opiate Analysis”

## Overview:

The Orange County Crime Lab (OCCL) recently evaluated IMCSzyme® to determine whether it would more accurately and efficiently detect drug use when compared to *Helix pomatia* β-glucuronidase, the enzyme in use at the lab. The purpose of the study was to optimize the parameters of IMCSzyme® so that hydrolysis would be completed in less than one hour with an amount of the enzyme that was cost effective.

## Materials and Methods:

The OCCL conducted their testing on porcine blood spiked at various concentrations with the five opiate glucuronides: morphine-3-β-D-glucuronide (M3G), morphine-6-β-D-glucuronide (M6G), codeine-6-β-D-glucuronide (C6G), hydromorphone-3-β-D-glucuronide (H3G) and oxymorphone-3-β-D-glucuronide (O3G) being studied and used standard operating procedure for opiate analysis. The blood, which was cleaned via SPE, was analyzed for opiate content by hydrolyzing 1mL of blood. The OCCL tested with 100 μL of IMCSzyme® in the 30 minute hydrolysis time as well as with 200 μL of IMCSzyme® in a one hour hydrolysis time.



Figure 1. Enzyme solutions from left: *Helix Pomatia*, Abalone, IMCSzyme®

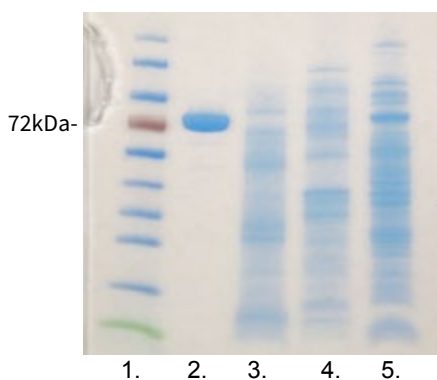


Figure 2. Gel imaging of various enzyme sources

1. Molecular weight ladder
2. IMCSzyme®
3. Abalone
4. *Helix Pomatia*
5. *E. coli*

## Results:

The OCCL studies showed that hydrolysis of the five glucuronide forms could be achieved using 100 μL of IMCSzyme® with a 30 minute hydrolysis time at 55°C. \* % Error =  $\frac{|Target\ concentration - experimental\ concentration|}{Target\ Concentration} \times 100$

## Conclusions:

IMCSzyme® allowed the OCCL to use a smaller amount of enzyme during testing, which was more cost effective, increased sample throughput and also saved time. The OCCL's experiments also showed that IMCSzyme® provided a more complete hydrolysis than *Helix pomatia* β-glucuronidase, which they had previously been using. OCCL was processing an equivalent of 10,340 toxicology exams for opiates that required three hours of hydrolysis before they began to use IMCSzyme®. Not only does IMCSzyme® allow them to conduct a total opiate analysis in a single day, but it is estimated that IMCSzyme® can save the OCCL up to 20,680 hours in testing time each year.

Table 1. 30 minute hydrolysis

Drug	Target Concentration (μg/mL)	Amount of Enzyme (μL)	% Error*
M3G	1.5	100	1.5
		200	35.5
	0.05	100	9.2
		200	15.2
M6G	1.5	100	4.6
		200	27.8
	0.05	100	-5.8
		200	16
C6G	2	100	-12.9
		200	0.1
	0.05	100	-39.9
		200	-31.4
H6G	0.8	100	7.2
		200	12.5
	0.05	100	6.8
		200	11.0
O3G	1	100	39.8
		200	9.1
	0.02	100	23.8
		200	-8.3

Table 2. 1 hour hydrolysis

Drug	Target Concentration (μg/mL)	Amount of Enzyme (μL)	% Error*
M3G	1.5	100	-10.8
		200	-9.7
	0.05	100	-0.6
		200	11.9
M6G	1.5	100	-3.2
		200	12.3
	0.05	100	3.4
		200	23
C6G	2	100	-1.0
		200	9.4
	0.05	100	-9.9
		200	117
H6G	0.8	100	-7
		200	5.3
	0.05	100	0.6
		200	2.8
O3G	1	100	21.1
		200	9.5
	0.02	100	151.5
		200	-18.8