Are You Paying Extra Money to Waste Enzyme?

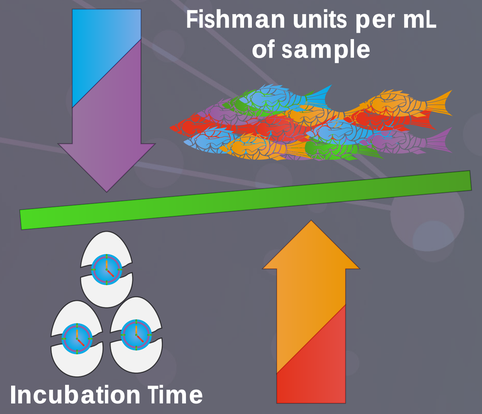
Introduction:

There is a heavy burden placed on toxicology labs to not only produce accurate and concise results, but to do it in a short period of time. Some processes can be altered or even omitted in order to save time, but some steps cannot be modified. Hydrolysis of glucuronides is a necessary step during sample preparation in order to accurately detect and quantify the amount of native, parent drug in a patient's system. Today's market offers a plethora of enzymes for analysts to choose from, which means, they must not only consider the source of the enzyme, but also recommended volumes, incubation times, and overall handling conditions. All of these things have an effect on cost per sample moving targets.

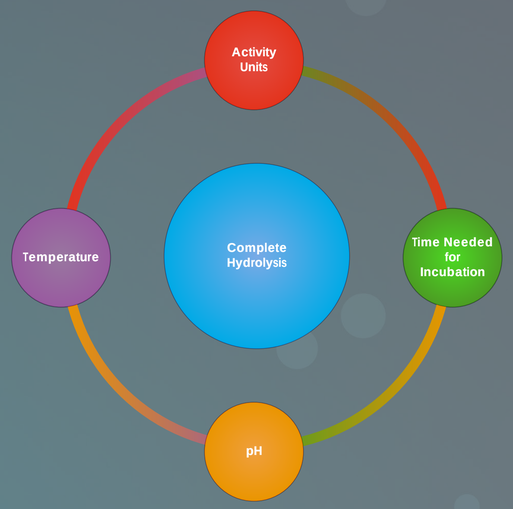
Recently, there has been a lot of debate as to what form of enzyme works most efficiently. Common questions of debate include:

1. Do the enzymes that undergo a purification process function better?
2. Are the more expensive, fully recombinant enzymes top performers?
3. Is there a maximum threshold to how much enzyme/activity units can be added before the enzyme itself plateaus?
4. What is the most optimal working temperature and pH to drive the hydrolysis reaction and does that differ via enzyme source and supplier?

Typical overall activity levels for various enzyme suppliers on the market range from 50,000 fishman units/mL all the way up to 180,000 fishman units/mL.



Common Variables that Dictate Hydrolysis Results



Most manufacturers supply suggested protocols along with the enzymes they sell, but do we really look into whether or not we are using too much enzyme? Overloading a sample will help drive the reaction forward, but where is the balance?

Experimental:

UCT's purified Abalonase™ and Abalonase™ Ultra were run along with an alternative supplier of fully recombinant β- glucuronidase. The activity of each of the enzymes and evaluated conditions were as follows:

Sample Prep

- 1 mL Blank Urine (fortified with standards)
- 1 mL Provided Buffer (diluted)
- 50 µL of Respective Enzyme
- Incubated 30 minutes at 60° C*

Total Units of Activity per Sample **

- Abalonase™ = 3,000 units/mL
- Abalonase™ Ultra = 9,000 units/mL
- Alternative Supplier = 4,850 units/mL

* Thirty-minute incubation time was chosen due to consistency of that recommended timeframe in various existing hydrolysis protocols.
** Based on actual activity units per manufacturer's COA's.

Results:

Conversion

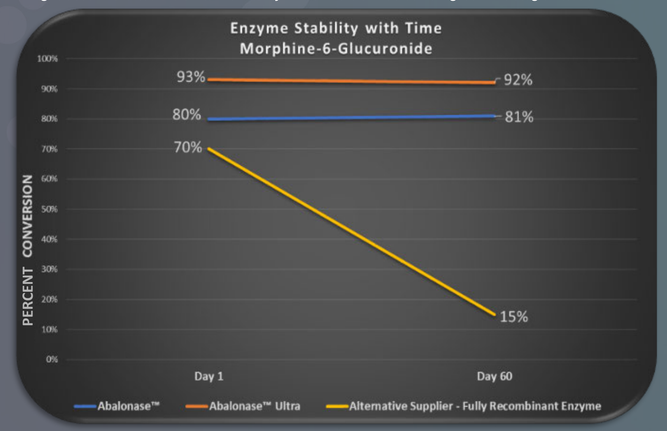
Conversion rates were calculated by fortifying drug free urine with both critical opiate glucuronides, morphine-6-glucuronide and codeine-6-glucuronide at a concentration of 100 ng/mL to liberate 62 ng/ml of each, respectively, upon complete hydrolysis.

Conversion Rate

Enzyme Type	% Conversion Morphine 6 Glucuronide (n=3)	% Conversion Codeine 6 Glucuronide (n=3)
Abalonase™	80	60
Abalonase™ Ultra	93	62
Alternative Supplier - Fully Recombinant Enzyme	70	35

Stability

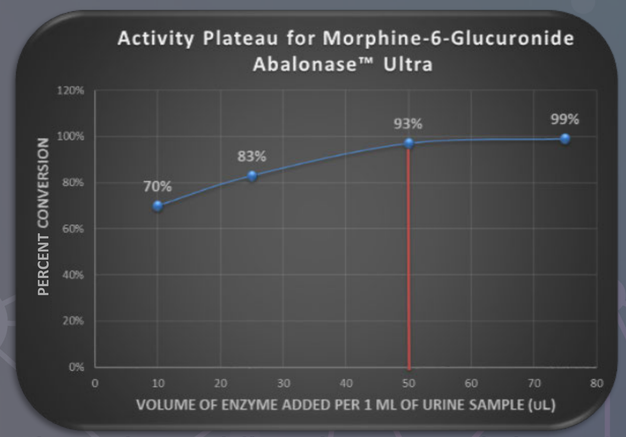
It has been documented that enzyme activity does decline sharply over time, sometimes as quickly as 30 days once opened, especially if a working solution is created. Calculating the required usage volumes within a window where activity decline won't be noted is a critical component to deciding what enzyme supplier to utilize. Both UCT's Abalonase™ and Abalonase™ Ultra remain stable over time providing customers additional flexibility when it comes to long term usage rates.



Is Less Enzyme More?

Several available hydrolysis protocols referenced in the literature recommend adding exorbitant amounts of enzyme to urine samples, sometimes even at an upwards of a 1:1 ratio. Depending on the analyte of interest, following the "flooding technique" does not necessarily drive the reaction to completion any further than if using a more conservative, fixed amount. In addition, by identifying where your particular enzyme supply plateaus, it can help control internal costing per sample and allow for extended usage. For example, a 10 mL bottle of enzyme can hydrolyze the following amount of samples based on volume added:

50 µL/sample → 200 samples
75 µL/sample → 133 samples
100 µL/sample → 100 samples



Why use that much if you don't necessarily have to?

Conclusion:

- Fully recombinant forms of the enzyme have proven to work very well for labs all over the world. However, if you look at the literature, you will notice that in order to get these excellent conversion rates in short periods of time you must overload your sample with enzyme.
- Adding high amounts of enzyme will help drive the reaction forward regardless of what type of enzyme you use, but it will also drive up the cost per sample for the lab.
- Why pay extra for modified enzymes if you can do the same thing for less money?
- Understanding the optimal conditions for your chosen enzyme will not only help you to maximize your enzymes efficiency, but also allow you to get the most productivity/lifetime out of your supply.