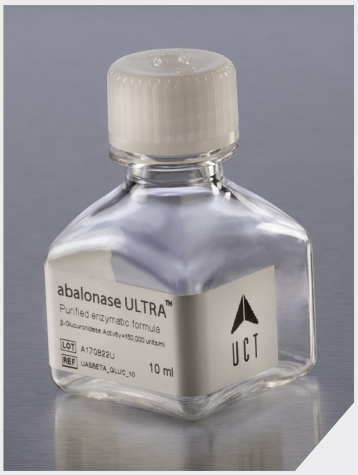
FORENSICS

UCT

UCT Enzymes at a Glance & Commonly Asked Questions



Enzyme Type	Abalonase™	Abalonase™ +	Abalonase™ Ultra	Selectrazyme® (Liquid)	Selectrazyme® (Powder)
Units of Activity	50,000 units/mL	50,000 units/mL	150,000 units/mL	100,000 units/mL	1,000,000-3,200,000 units/gram
Purified	YES	YES	YES	NO	NO
Hydrolysis Buffer Included	YES	YES	YES	NO	NO
Optimal Hydrolysis pH	4.5	5	4.5	5	5
Stability *	1 YEAR	1 YEAR	1 YEAR	1 YEAR	3 YEARS
Storage Conditions	2ºC to 8ºC	2ºC to 8ºC	2ºC to 8ºC	2ºC to 8ºC	- 20ºC
Average Hydrolysis Time * *	15-90 minutes	15-90 minutes	0-60 minutes	1-3 hours	1-3 hours
Added Sulfatase Activity	NO	YES	NO	YES-Minimal (Inherent secondary activity)	YES-minimal (Inherent secondary activity)

* When stored properly-unopened
** Compound dependant

ß-Glucuronidase Commonly Asked Questions

What is the source of UCT's enzyme?

Selectrazyme®, Abalonase™, Abalonase™+ and Abalonase™ Ultra enzymes are all derived from abalone (a type of shellfish). Abalone derived β-glucuronidase is reported to provide better hydrolysis efficiency for opiates, benzodiazepines and steroids compared to ß-glucuronidase derived from other species, including Helix Pomatia, Patella Vulgata, Haliotis Rufescens, E.Coli and bovine liver. In addition, abalone derived β-glucuronidase is more thermally tolerant compared to enzymes derived from other sources. This offers the advantage of carrying out the hydrolysis reaction at a higher temperature and shortens the incubation time required while achieving a higher degree of hydrolysis of the drug conjugate(s).

What is the difference between the Selectrazyme® and Abalonase™ product lines?

Both product lines use enzymes that are isolated from abalone. Selectrazyme® contains unpurified enzyme which is suitable for applications that utilize sample cleanup (e.g. SPE) prior to instrumental analysis. It is not suitable for direct injection (i.e. dilute-and-shoot). Abalonase™ is a purified β-glucuronidase formula that has been designed to quickly hydrolyze conjugated drug metabolites. Due to the purification, this enzyme is suitable for applications that will not undergo any additional sample purification prior to analysis (i.e. dilute-and-shoot). In addition to this,
Abalonase™ possess enhanced catalytic efficiency, where half the units of activity provide the same metabolic conversion rate as a traditional abalone derived enzyme.

What if I want to cleave sulfate bound drugs in addition to glucuronic acid bound drugs?

In this case you must use an enzyme that has secondary sulfatase activity. Selectrazyme® (liquid or powder form) and Abalonase™+ both contain glucuronidase and sulfatase activity and are able to cleave glucuronic acid and sulfate bound drugs (e.g. steroids).

What forms of enzyme are available from UCT?

Selectrazyme® is supplied in liquid and powdered (lyophilized) form. Abalonase™ is only available in liquid form. The enzymes are supplied at various concentrations (units per mL, U/mL).

Stock enzyme:
Selectrazyme® liquid enzyme activity = ≥100,000 U/mL
Selectrazyme® lyophilized enzyme activity = 1,000,000 - 3,200,000 U/g
Abalonase™ = ≥ 50,000 U/mL
Abalonase™+ = ≥ 50,000 U/mL (β-glucuronidase) & 400 U/mL (sulfatase)
Abalonase™ Ultra = ≥ 150,000 U/mL

What is the optimal units of activity per mL of urine sample?

Selectrazyme® = 5,000 U/mL
Abalonase™ = 5,000 U/mL
Abalonase™+ = 5,000 U/mL (β-glucuronidase) & 400 U/mL (sulfatase)
Abalonase™ Ultra = 15,000 U/mL

These are the optimum units of activity recommended for UCT's enzymes. Depending on the application and the specific drug conjugates to be hydrolyzed, alternative amounts of enzyme

(U/mL) may be used at the customer's discretion. In this case, acceptable performance of the enzyme cannot be guaranteed.

What is the optimum pH and concentration of buffer to use for hydrolysis?

The optimum pH for the Selectrazyme® enzyme is pH 4.0 (β-glucuronidase) or pH 5.0 (β-glucuronidase and sulfatase). The optimum concentration of the buffer solution is 0.1M. An acetate or citrate buffer can be used.

Abalonase™, Abalonase™+ and Abalonase™ Ultra are each supplied with a specific Rapid Hydrolysis Buffer for optimum performance (for more details see next question). The working pH range of the Abalonase™ product line is pH 4.2–5.5. The Rapid Hydrolysis Buffer is supplied as a 1.0 M solution but the working concentration (after dilution and mixing with sample) is 0.1M.

What is the Rapid Hydrolysis Buffer comprised of?

Abalonase™ , Abalonase™ + and Abalonase™ Ultra are supplied with Rapid Hydrolysis Buffer. Through the use of the provided buffer the enzymes will achieve their optimum performance. In addition, it will significantly reduce overall sample preparation times.

The Rapid Hydrolysis Buffer that is included with Abalonase™ and Abalonase™ Ultra is comprised of a sodium acetate buffer at pH 4.5 (1.0 M). The Rapid Hydrolysis Buffer that is included with Abalonase™ + is pH 5.0 which is the optimum pH for the combined β-glucuronidase and sulfatase activity. The buffers are sterilized and filtered through a 0.2 µm membrane to ensure no interfering contaminants are present. All the ingredients in the Abalonase™ , Abalonase™+, Abalonase™ Ultra formulas and buffers are compatible with mass spectrometry.

What is the stability of the enzyme?

The liquid enzyme and buffer are very stable when stored separately at 4°C. Stability typically only becomes an issue when the two are combined to create the working enzyme/buffer mix. When properly stored, the enzyme will maintain activity for at least 1 year. The buffer should be stable up to 6 months if stored at 4°C (however, check periodically for any bacterial growth). The working enzyme/buffer mix can be stable for up to 1 week (4°C). However, to minimize any stability issues and avoid potential problems it is suggested to prepare the enzyme/buffer mix on a daily basis.

Lyophilized powder, when stored correctly at -20°C, will maintain activity for at least 3 years. Once diluted with buffer it will have similar stability to the liquid enzyme mentioned above.

It is always best practice to store any enzyme, buffer and enzyme/buffer mix at 4°C when not in use. This will ensure maximum stability.

Can the enzymes be frozen and then thawed for use?

The liquid form of the enzyme (stock enzyme or working solution) will be negatively affected if frozen and thawed. This is due to the formation of ice during freezing which damages the protein structure of the enzyme. This will result in a decrease in enzyme activity. Therefore, the liquid form of the enzyme should never be frozen. Lyophilized (powdered) enzyme is stable when stored in a freezer at -20°C.

When preparing the enzyme, do I need to add all of the Abalonase™ enzyme to the Rapid Hydrolysis Buffer?

No, the enzyme and buffer can be stored separately and combined as needed. In this case the concentrated vial of Rapid Hydrolysis Buffer can be diluted with DI H_2O (as outlined in the respective Hydrolysis Protocol) and stored separately from the enzyme. The buffer should be stable up to 6 months if stored at 4-8°C (however, check periodically for any bacterial growth). Appropriate volumes of each the stock enzyme and diluted buffer solution can then be combined on a daily basis or as needed.

If all of the enzyme and Rapid Hydrolysis Buffer are combined then this working solution will be stable for up to 1 week. However, to minimize any stability issues, it is highly recommended to prepare the enzyme/buffer mix on a daily basis if it is not going to be used within a few days.

For the Abalonase™ enzymes, the time range for incubation varies from almost instantly to 3 hours. Why such a wide range?

The time range listed accounts for a wide variety of compounds to be hydrolyzed. For example, benzodiazepines like oxazepam and lorazepam are rapidly hydrolyzed and require little to no incubation time. The more difficult compounds such as opiates, morphine-6-glucuronide and codeine-6-glucuronide will take much longer, sometime up to 3 hours depending on how much enzyme you add and the incubation temperature. This is primarily due to steric hindrance which can hinder the enzymes ability to access and cleave the functional groups on the metabolite.

How is recovery calculated?

In order to accurately determine hydrolysis effectiveness (i.e. recovery), the difference in molecular mass of the bound drug versus the free parent drug needs to be considered. This is calculated according to the following equation:

$$\text{Concentration of free parent drug (ng/mL)} = \text{Concentration of glucuronide metabolite (ng/mL)} \times \frac{\text{Molecular weight of free parent drug (g/mol)}}{\text{Molecular weight of glucuronide metabolite (g/mol)}}$$

For example, the molecular weight of Lorazepam is 321.2 g/mol and Lorazepam-glucuronide is 497.3 g/mol. If a Lorazepam-glucuronide control sample is prepared at 100 ng/mL and complete hydrolysis is achieved (100% conversion of Lorazepam-glucuronide to lorazepam), then the final concentration of the free parent Lorazepam would be 64.6 ng/mL (100 ng/mL × 321.2 g/mol ÷ 497.3 g/mol). Recovery should be calculated based on this value (in this case 64.6 ng/mL Lorazepam following incubation equals 100% conjugate conversion back to parent not 64.6% conversion.

If you would prefer to calculate the recovery based off a final concentration of 100 ng/mL Lorazepam then more Lorazepam-glucuronide would need to be added to the control sample at the beginning (in this case 154.8 ng/mL).

This applies to any bound drug's mass versus the parent mass, including oxazepam glucuronide, morphine-3 glucuronide, morphine-6 glucuronide, codeine-6 glucuronide, etc.

All calculations must be done independently