B-One® QUICK START GUIDE

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Product Overview: **B-One®** is a 3rd-generation recombinant β-glucuronidase delivered in a stabilized, "All-in-One" reaction buffer. Designed for room-temperature, high-throughput automated workflows, it eliminates supplemental buffering, reagent mixing, and heating steps. **B-One®** provides high hydrolytic activity against a broad spectrum of drug classes, including hard-to-cleave conjugates like codeine-6-glucuronide.

Working Temperature Range: Room Temperature (19-22 °C)

Working pH Range: 6.8 - 7.2

Purity: ≥ 98% enhanced recombinant β-Glucuronidase

Storage: 18 months when stored at 2–8°C. Alternatively, up to 3 months when stored at 19–22°C, regardless of whether the vial is opened or unopened.

Enzymatic Activity: ≥12,000 PS-U**

Buffer: No Supplemental Buffering needed.

Table 1: B-One® recommended reaction conditions

Enzyme to Sample Ratio	**Comprehensive panel with Opiates/Opioids**	**Benzodiazepines**	**Cannabinoids**
Urine : B-One® (v/v)	1:1	5:2	5:3
Example of Recommended Hydrolysis Mix			
Component	**Comprehensive panel with Opiates/Opioids (µL)**	**Benzodiazepines (µL)**	**Cannabinoids (µL)**
Urine*	50	50	50
B-One®*	50	20	30
ISTDs (100% MeOH)*	10	20	20
DI-H2O	40	60	20
Total Volume	**150**	**150**	**120**
Incubation at RT	**15 minutes**	**5 - 10 minutes**	**Instantaneous**

Component volumes can be modified for specific sensitivity requirements. However, it is suggested to keep the Urine : **B-One® ratios and MeOH concentration between 5-15% in the final mix for optimal enzymatic performance.*

Hydrolysis Protocol:

1. Optional: Centrifuge urine sample for 5 minutes at 20,000 x g and/or dilute at least 3 times to reduce the possible impact that inhibitors might have on the optimal enzyme performance.
2. With a pipette, add 50 µL urine samples to a test tube or 96-well plate according to Table 1.
3. Add Internal Standards (ISTDs) + **B-One®** + DI-H_2O (if applicable) to the urine sample as shown in Table 1.
4. Mix slowly by pipetting up and down. If an automated pipetting station is used, mixing can be done by repeating the aspirate/dispense action.
5. Incubate for the indicated time at room temperature.
6. Proceed with the preferred extraction method: For a dilute and shoot (D&S) method, add the curve-diluent and inject; or proceed with filter and shoot (F&S), solid-phase extraction (SPE), supported liquid extraction (SLE), liquid-liquid extraction (LLE), or protein crash.

Important Notes:

- **Gently mix before use**; **do not shake or vortex.**
- Enzymatic hydrolysis with **B-One®** is compatible with various sample preparation methods such as D&S, F&S, protein precipitation (PPT), SPE, SLE, and LLE.
- **B-One®** is active in the presence of MeOH in a range from 0-30%, but is optimal between 5-15%.
- **B-One®** can be premixed with ISTD, reaching up to 30% MeOH or up to 10% ACN, and stored refrigerated for up to 14 days, contingent upon internal standard stability. If higher organic content is needed, contact us for recommendations.

**One PS-U will liberate 1.0 µg of phenolphthalein from phenolphthalein glucuronide in 5 minutes at pH 6.8 and 25°C.

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