



FAQs

Sample and Loading

Q: How much sample can I process per well or 1 mL tube?

A: You can process up to 200 μ L of urine hydrolysate – this sample is then diluted with 133 μ L of methanol, which will result in a total loading volume of 333 μ L in each case. (Note: The 133 μ L of methanol is equivalent to a 40 % dilution.)

Q: Do I have to use the 40 % methanol dilution?

A: Yes – However, it is possible to load 200 μ L of the urine hydrolysate (undiluted), followed by a “secondary elution” of 133 μ L of methanol to achieve the same results. If you want to use less than 200 μ L of urine lysate, employ the following formula to determine how much methanol you need for dilution:

$$\mu\text{L Methanol} = (2/3) * (X \mu\text{L Urine Hydrolysate})$$

Loading less than 100 μ L of urine hydrolysate is not recommended.

Q: How much sample can I expect to get out of the filter?

A: β -Gone tubes and 96-well plates for recombinant enzymes contain 30 mg of sorbent and have a corresponding dead volume of \sim 75 μ L while the β -Gone tubes and 96-well plates for non-recombinant enzymes contain 45 mg of sorbent and their dead volume is approximately \sim 100 μ L. For information on how to appropriately calculate recovery, please see “How do I calculate recovery” below.

Extraction and Recovery

Q: How do I calculate recovery?

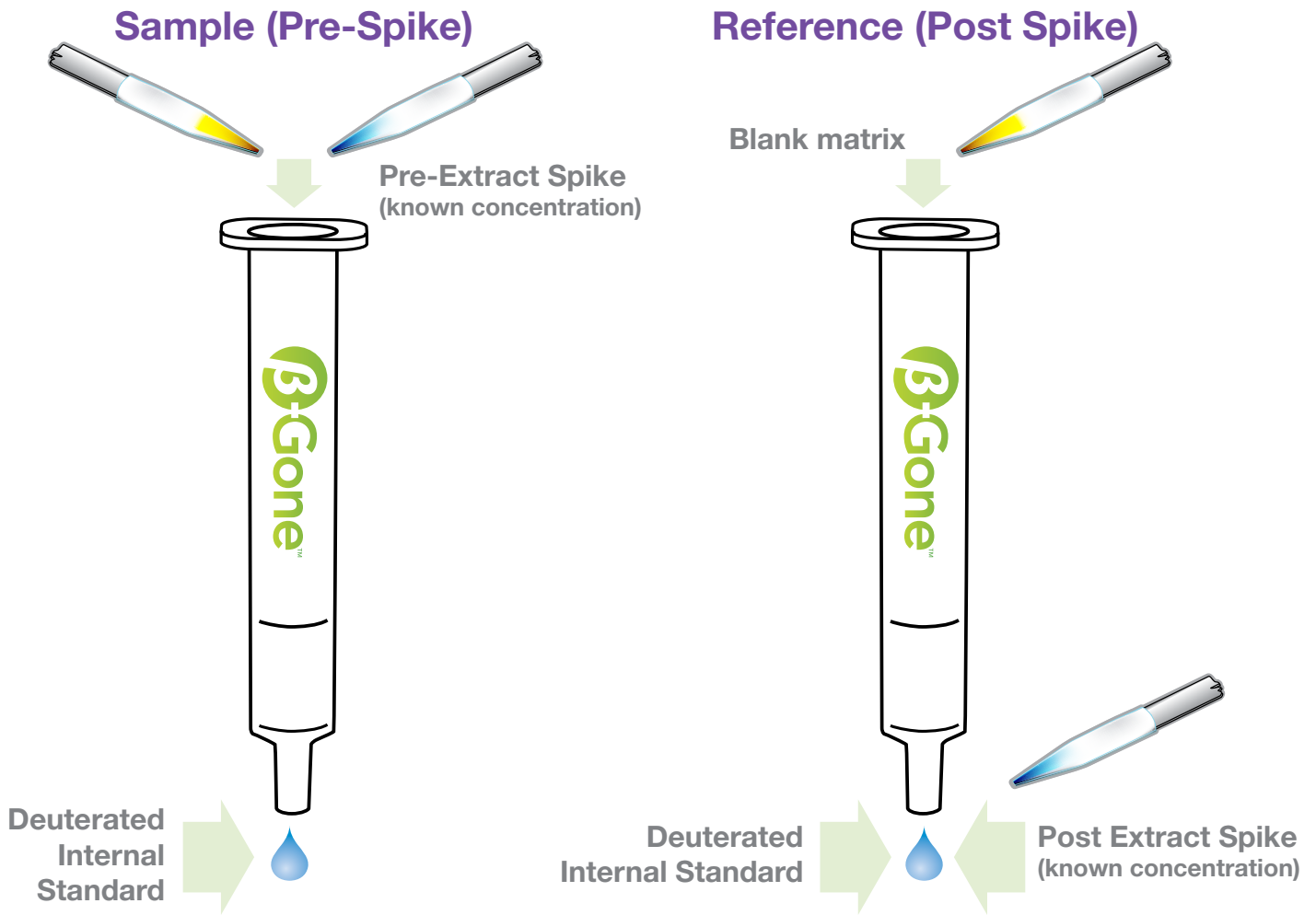
A: For assessing the performance of this product, it is recommended that you calculate Absolute Recovery. Absolute Recovery is defined as follows:

$$\text{Absolute Recovery} = \frac{\text{Pre-Extract Spike Peak Area}}{\text{Post-Extract Spike Peak Area}} \times 100$$

Or sometimes stated as:

$$\text{Absolute Recovery} = \frac{\text{Sample}}{\text{Reference}} \times 100$$





There can be variable amounts of reference sample (blank matrix) lost to dead volume depending on vacuum setting and time spent with vacuum open after passing the sample through the plate/tube. In order to make sure that the Post Extract Spike is spiked with the appropriate level of analyte (i.e. to match the concentration of the pre-spike) we suggest implementing the following technique:

Remove exactly 150 μ L of the reference pass through and spike that to the same concentration as the pre-spike.

Please contact support@phenomenex.com if you would like to learn more about β -Gone and our other sample preparation products.

