

# Sim 10 Pouch Sample Preparation

## Introduction

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Sample preparation should be done before reagent preparation. Each DNA sample must be in liquid form before running PCR and have a minimum volume of 500  $\mu\text{L}$  (0.5 mL). Sample preparation is essential, because samples must be free of any contaminating salts or other compounds that would disrupt or inhibit the enzymatic reactions and fluorescent dyes required to run the reaction. Before beginning to sample, label all unknown tubes with unique identifiers (i.e., Sample 1).

## Simulant Preparation

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### Yeast (*Saccharomyces cerevisiae*)

1. Purchase a packet of baker's yeast (such as Fleischmann's<sup>®</sup> or Red Star<sup>®</sup>) from a local supermarket. Use as is for a powder sample and follow the Dry Sample protocol below.
2. To prepare a liquid sample, take 10–50 grains of the yeast powder and add to ~500  $\mu\text{L}$  (.5 mL) of reagent grade water. Shake to mix the sample and follow the Liquid Sample protocol below.

### *Bacillus globigii* (Bg)

1. Obtain a dry sample of Bg. Follow the Dry Sample protocol below.
2. To use a liquid sample, dilute 1 mg of Bg in 5 mL of reagent grade water and follow the Liquid Sample protocol below.
3. To process an aerosolized sample of Bg, follow your company's Standardized Operating Procedure (SOP) for collecting an air sample using an air sampling collection system. We recommend collecting into reagent grade water then following the Liquid Sample protocol below. Call ITI if you have questions about potential inhibitors contained in your buffer solution.

## Sampling

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### Dry Sample (Powders, etc.)

1. When sampling a visible contamination, i.e., concentrated sample pile, touch the dry swab to the unknown powder with one full rotation of the swab. If sample does not adhere to the dry swab, dip the swab briefly into the Unknown Sample Vial and touch the wet swab to the unknown powder with one full rotation of the swab.
2. If the contamination is spread over a large area, sample a 2" x 2" concentrated area by first dipping the swab briefly into the Unknown Sample vial and swiping the unknown powder, residue, or contaminated surface with a sweeping motion on both sides of the moist swab.

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**Note:** Make sure to collect a “representative sample” of the area by swiping the middle of the area as well as the edges. If the powder appears to contain different particles, make sure you swipe each.

3. Place the swab into the Unknown Sample vial and break off at the break point.



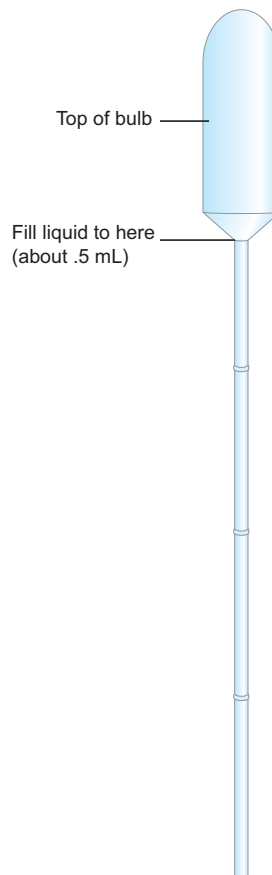
4. Secure the cap on the vial and shake vigorously for 30 sec.
5. Repeat any remaining samples using a fresh swab and new Unknown Sample vials.

#### Liquid Sample (Air samplers, cultures, etc.)

1. Transfer approximately 0.5 mL of liquid to the Unknown Sample vial.

**Note:** To draw a sample with the transfer pipette, first squeeze and hold the top of the bulb. Insert the tip of the pipette into the liquid and release the bulb to draw sample up to the fill line (see illustration). Transfer the pipette to the vial and squeeze the bulb to empty the sample into the vial.

2. If sampling from a large body of liquid, make sure to collect a “representative sample” by collecting liquid samples from separate areas (entry point, middle, edges, etc.)
3. Secure the cap on the Unknown Sample vial and shake vigorously for 30 sec.
4. Repeat any remaining samples using a fresh pipette and new Unknown Sample vials.
5. Load the diluted Unknown sample(s) into a RAZOR™ pouch following the instructions found in the “Loading Reagent Pouches” section in the *RAZOR Pouch Instruction Booklet*.



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## Dilution

For a sample that might contain a high level of inhibitors, a dilution step may be necessary. This would be indicated if the inhibition control in the pouch failed on the first RAZOR run of an unknown sample. To perform a dilution on dry or liquid sample, follow these steps:

1. Use sample previously created in the Unknown Sample vial or add sample to a vial as described in the previous two sections.
2. Using a new pouch kit, transfer approximately 0.5 mL from the original vial to another labeled vial (i.e., Diluted Sample 1) using a transfer pipette.
3. Secure the cap and shake vigorously for ~30 sec.
4. Load diluted samples into a pouch using the “Loading Reagent Pouches” section in the *RAZOR Pouch Instruction Booklet*.

**Once the raw sample has been prepared properly, load the sample into the pouch.**

For more instructions on preparing dry and liquid samples, please see either the specific pouch assay card protocol or the *RAZOR Pouch Instruction Booklet*.

*Questions? Contact ITI Technical Support*  
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support@idahotech.com

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