

Protocol for Whole Bacterial Isolation in the Bullet Blender™

The protocol described in this document is for the use of the Bullet Blender™ for the isolation of whole bacterial cells from organ tissue (this particular protocol was developed using kidney, lung and spleen, but can be used for other organs as well). While this protocol specifies PBS as the homogenization buffer, you may choose which is most appropriate for your downstream application.

Materials Required: organ tissue, Bullet Blender™, PBS, aspirator, pipettor, microcentrifuge tubes, and [3.2mm stainless steel beads \(part number SSB32\)](#).

Instructions

1. Cut the organ tissue into appropriately sized pieces for analysis (50mg-300mg) and place into microcentrifuge tube.
1. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes some external contaminants (blood, etc.).
2. Add a mass of stainless steel beads (3.2mm) equal to approximately 4x the mass of sample (each bead is approximately 140mg).
3. Add 300µl of PBS into the tubes.
4. Close the tubes.
5. Place in the Bullet Blender™.
6. Set the controls for **SPEED 8** and **TIME 4** minutes. Press **Start**.
7. Take out the tubes and inspect for complete homogenization of organ. If homogenization is unsatisfactory, run for another five minutes at **SPEED 8**.
8. Add an additional 200µl of PBS to tube. Set the controls for **SPEED 2** for 1 minutes to vortex your samples.
9. Proceed with your downstream application.

SAFETY NOTE!!!

When using a centrifuge to separate your homogenate from the debris and beads, make sure your tubes are balanced.

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