

# Protocol: Pharyngeal/Laryngeal tissue Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of Pharyngeal/Laryngeal tissue. This protocol was created using oropharyngeal tissue from humans. Other types of pharyngeal/laryngeal tissue or pharyngeal/laryngeal tissue from other species may require a slightly modified homogenization protocol. This protocol does specify a buffer, and there are additives to assist in tissue disruption, however it is likely that tissue could be homogenized without enzymatic or chemical assistance if the homogenization time was increased.

**Materials Required:** Pharyngeal/Laryngeal tissue, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes, and Navy bead lysis kit/Green bead lysis kit/0.9-2.0mm stainless steel bead blend (product number SSB14B).

## Instructions

1. Cut pharynx/larynx into appropriately sized pieces for analysis (10mg-300mg).
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, etc.).
3. a. *Samples 50mg or greater*  
Place the sample in Navy bead lysis kit tube.  
b. *Samples less than 50mg*  
Place the sample in Green bead lysis kit tube.  
c. *Alternate protocol step for bulk beads*  
Place sample in microcentrifuge tube and add beads to the tube. Use a volume of beads equal to the mass of tissue. **NOTE:** 100mg  $\approx$  100 $\mu$ L.
4. Add digestion buffer (10mM Tris, pH 7.5; 10mM EDTA; 0.5% SDS; 200  $\mu$ g/ml Proteinase K). Add two volumes of digestion buffer for each mass of tissue (for example, with 100mg tissue, use 200  $\mu$ l buffer)
5. Close the microcentrifuge tubes tightly, and place the tubes into the Bullet Blender®.
6. Set controls for **SPEED 10** and **TIME 5** minutes. Press start.
7. Remove tubes from the instrument.
8. Visually inspect samples, if homogenization is unsatisfactory, run for another three minutes at **SPEED 10**.
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.**

## Reference:

Winder, D.M., Ball, S.L.R., Vaughan, K., Hanna, N., Woo, Y.L., Fränzer, J., Sterling, J.C., Stanley, M.A., Sudhoff, H., Goon, P.K.C. [Sensitive HPV detection in oropharyngeal cancers](#). BMC Cancer 9(440): 2009

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