

Protocol: Pharyngeal 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 tissue. This protocol was created using oropharyngeal tissue from humans. Other types of pharyngeal tissue or pharyngeal 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 tissue, Bullet Blender™, homogenization buffer, pipettor, microcentrifuge tubes, and [0.5mm stainless steel beads \(part number SSB05\) or stainless steel bead blend \(part number SSB14B\)](#).

Instructions

1. If larger than approximately 100mg, cut tissue into pieces or approximately 100mg or smaller.
2. Place tissue into microcentrifuge tubes. No more than 300mg of tissue should be placed in a single tube.
3. Add stainless steel beads to the tubes. Use 3 times the mass of the sample (approx. ½ the volume of the sample) in beads.
4. Add digestion buffer (10mM Tris, pH 7.5; 10mM EDTA; 0.5% SDS; 200 µg/ml Proteinase K). Add two volumes of digestion buffer for each mass of tissue (for example, with 100mg tissue, use 200 µl 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