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 ≅ 100µL.
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

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