Protocol for Dermal Tissue / Skin Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of dermal tissue / skin. This protocol was developed for the homognization of human skin from the jaw area – skin from different areas of the body and especially from different species may differ in consistency and therefore require altered homogenization protocols. Particularly tough skin samples may require enzymatic pretreatment with collagenase and / or hyaluronidase in order to achieve good homogenization. This protocol does not specify a particular buffer - you may choose which is most appropriate for your downstream application (nucleic acid isolation, protein extraction, etc.).

Materials Required: dermal tissue, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes, and Navy bead lysis kit/0.9-2.0mm stainless steel bead blend (product number SSB14B), 3.2mm stainless steel balls*

Instructions

1. If your tissue is larger than 50mg, cut the skin tissue into long, thin strips of approximately 50mg in size or less.
2. OPTIONAL: Add 1mL hyaluronidase (H-3506, Sigma Chemical, St. Louis, MO) to sample and incubate (15 minutes at 37°C, on Next Rocker). Wash the sample with 1mL PBS. Centrifuge at 1000g for 5 minutes.
3. OPTIONAL: Add 1mL collagenase, type II (CLS2, Worthington, Lakewood, NJ) to sample and incubate (2 to 4 hours at 37°C, on Next Rocker). Wash the sample with 1mL PBS. Centrifuge at 1000g for 5 minutes. Aspirate supernatant.
4. a. *Protocol step using pre-loaded tubes*
   Place the sample in Navy bead lysis kit tube.
   b. *Alternate protocol step for bulk beads*
   Place sample in microcentrifuge tube and add 50 to 100µl of the 0.9-2.0 stainless steel bead blend to the tube. For improved homogenization, add 1-5 3.2 mm stainless steel balls in addition to the stainless steel blend.*
5. Add 2 volumes of buffer for every volume of beads.
6. Close the microcentrifuge tubes and place the tubes into the Bullet Blender®.
7. Set controls for SPEED 10 and TIME 5 minutes. Press Start.
8. After the run, remove tubes from the instrument and inspect the samples.
9. If homogenization is unsatisfactory, run for another five minutes at SPEED 10.
10. If necessary, add more extraction buffer.
11. 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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