

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 homogenization 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 was created for the isolation of RNA and uses an RLT / β -mercaptoethanol reagent, however you may choose any buffer 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 [0.5mm or 0.9-2.0mm stainless steel beads \(part no. SSB05 or SSB14B\)](#).

Instructions

1. If your tissue is larger than 50mg, cut the skin tissue into long, thin strips of approximately 50mg in size or less. Individually place each piece of tissue into a microcentrifuge tube.
2. **OPTIONAL:** Add 1mL hyaluronidase to sample and incubate (15 minutes at 37°C, on Next Rocker). Centrifuge at 1000g for 5 minutes.
3. **OPTIONAL:** Add 1mL collagenase, type II (2 to 4 hours at 37°C, on Next Rocker). Centrifuge at 1000g for 5 minutes. Aspirate supernatant.
4. Add stainless steel beads (0.5mm or 0.9 – 2.0mm blend) to the tube. Use a mass of beads equal to 3x your mass of tissue.
5. Add 2 volumes of RLT / β -mercaptoethanol solution for every mass of tissue (so for 50mg of skin, add 100 μ l of buffer).
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.

This protocol obtained RNA concentrations of over 300ng/ μ l with RIN over 8.5

SAFETY NOTE!!!

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

Acknowledgment

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