User-Developed Protocol:

Isolation of DNA from soft tissue using the TissueLyser and EZ1 DNA Tissue Kit

This procedure has been adapted by customers and is for the isolation of DNA from soft tissues using the TissueLyser and the EZ1 DNA Tissue Kit.* **The procedure has not been thoroughly tested and optimized by QIAGEN.**

The TissueLyser allows high-throughput, rapid and effective disruption of up to 48 tissue samples.

Note: This protocol has only been tested with 'soft' tissues (e.g., liver, spleen, thymus, heart, kidney, and brain) and may not work with 'hard' tissues (e.g., bone, teeth, and skin).

The following guidelines can be used for both fresh and frozen tissues, and for tissues stabilized with RNA*later*™ RNA Stabilization Reagent.

Reagents and materials to be supplied by the user

- 5 mm stainless steel beads (cat. no. 69989)
- TissueLyser Adapter Set 2 x 24 (cat. no. 69982)
- 2 ml Safe-Lock microtubes (Eppendorf, cat. no. 0030 120.094)

Important note before starting

- Use 10 mg tissue as starting material, increasing the amount if the protocol works satisfactorily.

Procedure

1. **Pipet 190 µl Buffer G2 (tissue lysis buffer) into a 2 ml Safe-Lock microtube.**
2. **Add one stainless steel bead to each tube. For best results, use 5 mm (mean diameter) stainless steel beads.**
3. **Add 10 mg tissue to the tube, and assemble the TissueLyser.**
4. **Homogenize on the TissueLyser for 20 s at 30 Hz. Do not exceed this time as it may result in DNA shearing.**
5. **Centrifuge the sample briefly to ensure that all the tissue debris is on the bottom of the tube.**
6. **Add 10 µl proteinase K to the tube.**

   Note: Add 40 µl proteinase K if using RNA*later* stabilized tissues.

* All disruption steps can also be performed using the Mixer Mill MM 300 without modification.

7. **Incubate for 56°C for 1 h in a shaker incubator.**
8. **Briefly centrifuge the 2 ml microtube to remove drops from inside the lid.**
9. **Transfer the clear lysate into a new 2 ml Sample Tube (supplied with kit).**

   Any undissolved material should be removed by centrifugation at 300 x *g* for 1 min and transferring the supernatant to a new sample tube.
10. **Proceed with the "Protocol for Isolation of Genomic DNA from Tissue" in the *BioRobot® EZ1 Genomic DNA Handbook* or *EZ1 DNA Handbook*.**

Ordering Information

Product	Contents	Cat. No.
TissueLyser — for high-throughput disruption of a wide range of biological samples		
TissueLyser (220–240 V, 50/60 Hz)	Universal laboratory mixer-mill disruptor, 220–240 V, 50/60 Hz	85220
TissueLyser (120 V, 50/60 Hz)	Universal laboratory mixer-mill disruptor, 120 V, 50/60 Hz	85210
TissueLyser (100 V, 50/60 Hz)	Universal laboratory mixer-mill disruptor, 100 V, 50/60 Hz	85200
Accessories		
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2.0 ml microcentrifuge tubes on the TissueLyser	69982
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
TissueLyser Single-Bead Dispenser, 5mm	For dispensing individual beads (5 mm diameter)	69965

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