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## E.Z.N.A.<sup>®</sup> Tissue Direct PCR Kit

Product Number	TQ2310-00
Purifications	20 preps
L1 Buffer	2.5 mL
L2 Buffer	500 µL
PCR Neutralization Buffer	2.5 mL
Direct PCR Buffer (10X)	300 µL
Instruction Manual	✓

**New in this Edition:** Proteinase K is now premixed with L2 Buffer eliminating the resuspension step prior to use. L2 Buffer can be stored at room temperature for 12 months.

### Storage and Stability

All components of the E.Z.N.A.<sup>®</sup> Tissue Direct PCR Kit are stable for at least 12 months from date of purchase when stored as follows. Store PCR Neutralization Buffer at 2-8°C. L2 Buffer can be stored at room temperature for up to 12 months. For long-term storage, store L2 Buffer at 2-8°C.

### User Provided Materials

- Water bath or heating block capable of 56°C
- Water bath or heating block capable of 95°C
- Nuclease-free 1.5 mL microcentrifuge tubes
- Microcentrifuge capable of 13,000 x *g*
- Vortexer

## Before Beginning

- Please take a few minutes to read this pamphlet thoroughly and become familiar with the protocol. Prepare all materials required before starting.
- Follow general lab protection procedures such as wearing gloves and safety glasses when handling any reagent supplied with the kit. Avoid contact with skin.
- The Tissue Direct PCR Kit has been optimized for use with Omega Bio-tek's Direct PCR Buffer (10X) included with this kit. PCR Buffer from other companies may or may not be compatible.

## Tissue Direct PCR Protocol

1. Cut 5-10 mg tissue, ear punch, or mouse tail sample and place into a 1.5 mL microcentrifuge tube.
2. Add 100  $\mu$ L L1 Buffer and 20  $\mu$ L L2 Buffer. Mix thoroughly by pipetting or vortexing.
3. Incubate for 10 minutes at 56°C.
4. Incubate for 3 minutes at 95°C. The tissue will not be completely digested during incubation. This is normal and will not affect performance.
5. Add 100  $\mu$ L PCR Neutralization Buffer to the lysate. Mix thoroughly by vortexing.
6. Centrifuge at 13,000*g* for 5 minutes at room temperature.
7. Transfer the supernatant to a new 1.5 mL microcentrifuge tube. Store at 4°C or use immediately in PCR reaction.
8. PCR amplification: Add 1-5  $\mu$ L lysate to 20-50  $\mu$ L PCR reaction using Direct PCR Buffer (10X). Add primers, Taq polymerase, dNTP, and MgCl<sub>2</sub> for PCR amplification.

**Example:** If doing a 50  $\mu$ L PCR reaction add 5  $\mu$ L Direct PCR Buffer (10X) and 1-5  $\mu$ L lysate.

**Note:** The volume of lysate should not exceed 10% of the total PCR reaction volume.