

Preparing Tissue Samples for DNA Purification

Materials to Be Supplied by the User

- deionized or Nuclease-Free Water (Cat.# P1193 or equivalent)
- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for incubation of samples (e.g., ClickFit Microtube, 1.5ml [Cat.# V1231])
- heating block set at 56°C

Note: This kit has been tested with tissue samples stored frozen (stored at –65°C or lower) prior to DNA purification. Completely thaw frozen samples before processing.

The total yield of genomic DNA from tissue samples depends on the tissue mass and type of tissue processed.

1. To lyse tissue samples, set the temperature of a dry heat block, water bath or thermal mixer to 56°C. Prepare incubation tubes that will fit in the desired heating option.
2. Transfer 5–50mg of tissue to each tube. Cutting tissue into smaller fragments may decrease lysis time. Centrifuge the tube at top speed for 15 seconds to collect the tissue pieces at the bottom of the tube.
3. Add 300µl of Nuclease-Free Water (Cat.# P1193 or equivalent) to each incubation tube.
4. Add 30µl of Proteinase K (PK) Solution to each incubation tube.
5. Vortex each tube for 10 seconds.
6. Add 300µl of Lytic Enhancer (LE2) to each incubation tube.
7. Vortex each tube for 10 seconds.
8. Incubate each tube at 56°C using one of the following options:
 - a. With a thermal mixer, use a high shake speed (e.g., 1,500rpm) for up to 2 hours.
 - b. With a dry heat block or water bath heater, use without shaking for at least 16 hours.
9. Vortex each tube for 10 seconds.
10. Centrifuge each tube at maximum speed in a microcentrifuge for 5 minutes to pellet any undigested material.
11. Transfer all of the supernatant from each incubation tube into a new tube. Avoid transferring any pelleted material. If a distinct fatty layer appears on top of the sample after centrifugation, do not transfer that layer to the new tube.
12. Add 300µl of Lysis Buffer to each new tube.
13. Vortex each tube for 10 seconds.
14. Prepare cartridges as described below.
15. Transfer each lysate sample from the incubation tube to well #1 (the largest well in the cartridge) of a separate cartridge and mix well with the binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture.

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Cartridge Preparation

1. Place the cartridge to be used in the deck tray with well #1 (the largest well in the cartridge) facing away from the elution position, which is the numbered side of the tray.
2. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing the cartridge in the instrument.
3. Add 15µl of RNase A Solution into well #3 of each cartridge.
4. Place a plunger in well #8 of each cartridge. Well #8 is the well closest to the elution tube.
5. Place an empty elution tube into the elution tube position for each cartridge. Add 50–200µl of Elution Buffer to the bottom of each elution tube.
Note: Use only the Elution Tubes (0.5ml) provided in the kit; other tubes may be incompatible with supported Maxwell[®] Instruments.
6. Follow the instrument run instructions in the *Maxwell[®] RSC Genomic DNA Kit Technical Manual #TM708*.

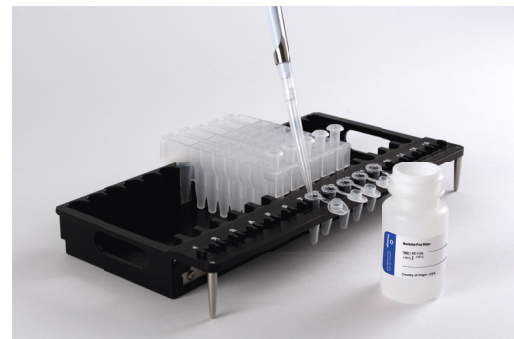


Figure 1. Setup and configuration of deck trays. Elution Buffer is added to the elution tubes as shown. Plungers are in well #8 of the cartridge. Deck tray shown is from the Maxwell[®] RSC Instrument (Cat.# AS4500).

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Additional protocol information is in Technical Manual #TM708, available online at: www.promega.com