Preparing Cell Pellet Samples for DNA Purification

Materials to Be Supplied by the User

- deionized or Nuclease-Free Water (Cat.# P1193 or equivalent)
- 1X phosphate-buffered saline (PBS) for cell pellet samples prepared from urine
- 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

Notes:

a. This kit has been tested with cell pellet samples from up to 5 × 10^6 cells processed immediately after generating a cell pellet and stored frozen (stored at –65°C or lower) prior to DNA purification. Completely thaw frozen samples before processing.

b. If sample freezing is desired, samples should be stored frozen after generating the cell pellet. Collecting a cell pellet from a sample that has been frozen and thawed can result in loss of performance.

The total yield of genomic DNA from cell pellet samples depends on the number of cells present in the sample.

1. Centrifuge the desired sample volume at a minimum of 2,000 × *g* for 20 minutes to generate a cell pellet.
   a. For urine samples, wash the cell pellet by resuspending in 750µl of 1X PBS.
   b. Centrifuge the PBS-suspended sample at a minimum of 2,000 × *g* for 20 minutes to generate a cell pellet.
2. Decant or aspirate the liquid from the pelleted cells. Resuspend the pellet in 300µl of Nuclease-Free Water (Cat.# P1193 or equivalent).
3. Prepare incubation tubes that will fit into the heating block.
4. Add 30µl of Proteinase K (PK) Solution to each incubation tube.
5. Transfer the desired sample volume (up to 300µl of 5 × 10^6 cells) to each incubation tube.
6. Vortex each tube for 10 seconds.
7. Add 300µl of Lytic Enhancer (LE2) to each incubation tube.
8. Add 300µl of Lysis Buffer to each incubation tube.
9. Vortex each tube for 10 seconds.
10. Incubate each tube in the 56°C heating block for 20 minutes. During this incubation, prepare cartridges as described below.
11. Vortex each tube for 10 seconds.
12. 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.

Maxwell® RSC Genomic DNA Kit

Instructions for Use of Product AS1880.

Quick Protocol

Maxwell® Automated DNA Purification

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 to 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**