Maxwell® RSC Fecal Microbiome DNA Kit

Instructions for Use of Product AS1700.

Quick Protocol

Preparing Fecal Microbiome Samples for DNA Purification

This Quick Protocol provides instructions for use of the Maxwell® RSC Fecal Microbiome DNA Kit with the Maxwell® Instruments to purify DNA human or mouse fecal samples. For detailed instructions, including information on instrument setup and troubleshooting, please refer to the Maxwell® RSC Fecal Microbiome DNA Kit Technical Manual #TM640.

Note: To use the Maxwell® RSC Fecal Microbiome DNA Kit, you must have the "Fecal Microbiome DNA" method loaded on the Maxwell® Instrument.

Materials to Be Supplied By User

- microcentrifuge tubes, 2.0ml
- sterile, aerosol-resistant pipette tips
- heat block
- microcentrifuge
- vortex

Preprocessing Fecal Samples

1. Place 100–300mg of fecal sample into a 2ml screw-cap microcentrifuge tube.
2. Add 1ml of Lysis Buffer and 40µl of Proteinase K to the microcentrifuge tube and vortex for 30 seconds.
3. Place the tube into a heat block at 95°C for 5 minutes.
4. Remove samples from heat block and allow to cool for 2 minutes on bench top.
5. Vortex thoroughly for 1 minute.
6. Incubate samples at 56°C for 5 minutes.
7. During the incubation (Step 6) prepare cartridges. See Preparing the Cartridge.
8. After incubation place lysate tubes into a microcentrifuge and spin at room temperature for 5 minutes at maximum speed (>10,000 × *g*) to pellet the solids.
9. Transfer only 300µl of supernatant into well # 1 of the reagent cartridge (Figure 1). Avoid pipetting any solid material from the bottom of the tube or on the surface of the liquid. Transferring these materials can inhibit downstream assays. If necessary, transfer the supernatant to a new tube and centrifuge again to avoid solids.
   Note: Some lysate will remain in the tube after transferring the 300µl aliquot to the cartridge.

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Automated DNA Purification

Preparing the Cartridge

1. Place the cartridges to be used in the deck tray(s) with well #1 (the largest well) facing away from the elution tube (Figure 1).
2. Press down on the cartridge to snap it into position. Carefully peel back the seal so the entire seal is removed from the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
   Caution: Handle cartridges with care. Seal edges may be sharp.
3. Place a plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
4. Place an empty Elution Tube into the Elution Tube position for each cartridge in the deck tray(s). Ensure that the caps are open and facing away from the cartridge positions
5. Add 100µl of Elution Buffer to the bottom of each Elution Tube.
   Notes:
   a. If Elution Buffer is on the side of the tube, elution may be suboptimal.
   b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may not work with the Maxwell® RSC Instrument.
6. Add 300µl of Binding Buffer to well #1 (the largest well) of each cartridge.
7. Add 20µl of RNase A to well #3 of each cartridge.
8. Return to Step 8 of Preprocessing Fecal Samples.

Figure 1. Set up and configuration of the deck tray.

Starting a Run on Maxwell® Instruments

Follow the instrument set up and run instructions in the *Maxwell® RSC Fecal Microbiome DNA Kit Technical Manual* #TM640.

Additional protocol information is in Technical Manual #TM640, available online at: **www.promega.com**

