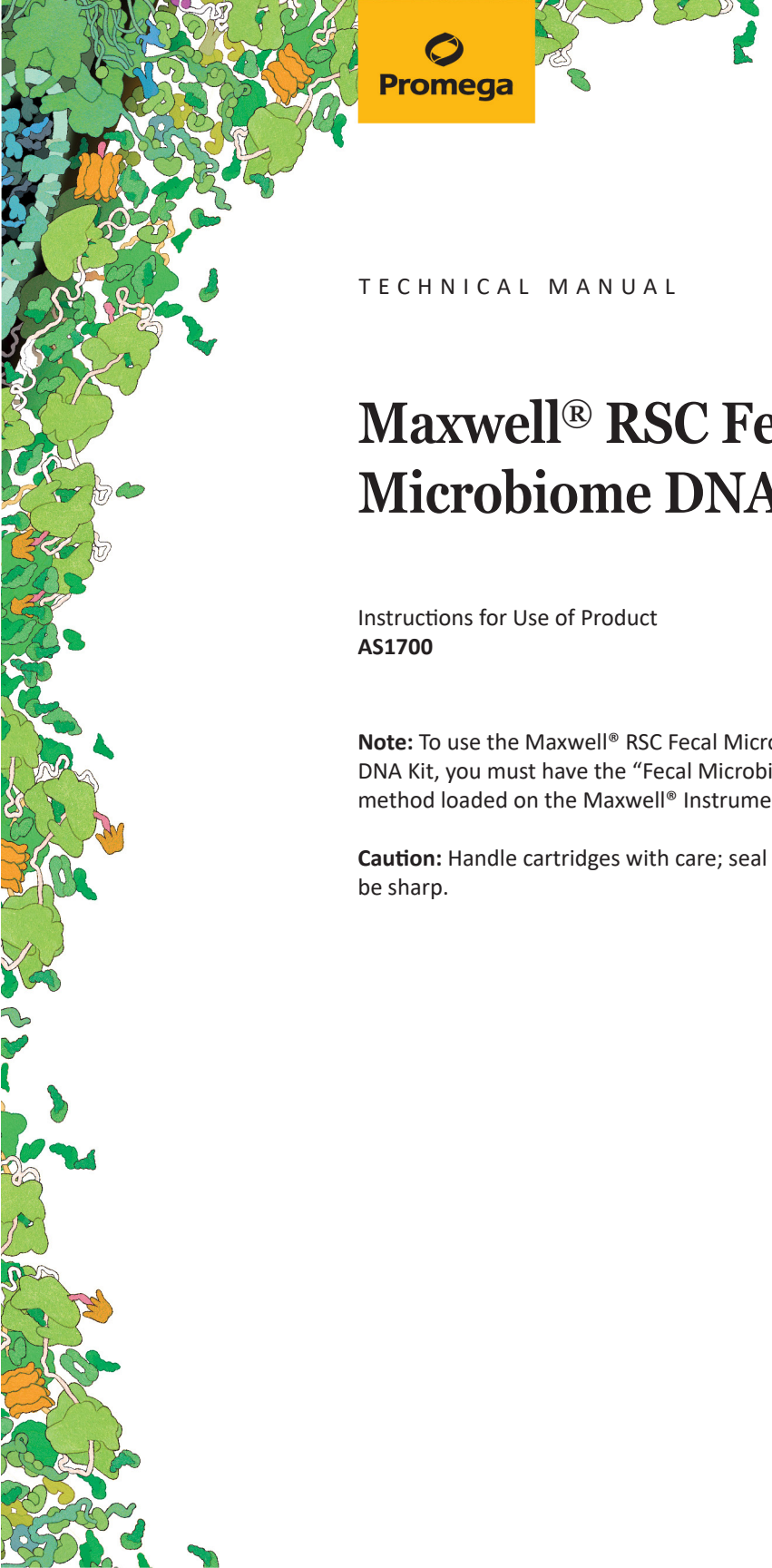

TECHNICAL MANUAL

Maxwell® RSC Fecal Microbiome DNA Kit

Instructions for Use of Product
AS1700

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

Caution: Handle cartridges with care; seal edges may be sharp.

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Promega

Maxwell® RSC Fecal Microbiome DNA Kit

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Fecal microbiome characterization is a rapidly evolving field based on continued development of downstream assays. Complex analyses such as next-generation sequencing can enhance data quality by measuring the abundance of various bacterial species. Many extraction methods for fecal microbiome DNA involve several manual processing steps and mechanical disruption. The Maxwell® RSC Fecal Microbiome DNA Kit[a], used with Maxwell® Instruments, is designed to provide an easy and automated method that efficiently purifies microbiome DNA for characterization in downstream assays including qPCR and next-generation sequencing.

The Maxwell® Instruments are designed for use with predispensed reagent cartridges, maximizing simplicity and convenience. Maxwell® methods for the Maxwell® RSC Fecal Microbiome DNA Kit can process up to 16 samples in approximately 60 minutes.

The Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind nucleic acids to the paramagnetic particles in the first well of a prefilled cartridge. The samples are processed through a series of washes before the nucleic acid is eluted. Using magnetic capture avoids common liquid-handling problems such as clogged tips or partial reagent transfers that result in suboptimal DNA extraction by other commonly used automated systems.

1. Description (continued)

The Maxwell® RSC Fecal Microbiome DNA Kit includes protocols for processing of samples with or without bead beating. A simple lysis without bead beating is sufficient for applications targeting abundant populations (Section 4.B). For users with applications needing more complete lysis, an optional bead beating lysis protocol is provided (Section 4.C). Several bead sets and conditions have been shown to be compatible with this purification workflow.

Table 1. Supported Instruments.

Instrument	Cat.#	Technical Manual
Maxwell® RSC	AS4500	TM411
Maxwell® RSC 48	AS8500	TM510
Maxwell® FSC	AS4600	TM462
Maxwell® CSC 48 (RUO Mode)	AS8000	TM628

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Fecal Microbiome DNA Kit	**48 preps**	**AS1700**

For Research Use Only. Sufficient for 48 automated isolations from fecal lysate samples. Includes:

- 100ml Lysis Buffer
- 20ml Binding Buffer
- 2 × 1ml Proteinase K (PK) Solution
- 2 × 1ml RNase A Solution
- 48 Maxwell® RSC Cartridges (RSCJ)
- 1 Maxwell® RSC Plunger Pack (48 plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® RSC Fecal Microbiome DNA Kit at +15°C to +30°C.

Safety Information: The reagent cartridges contain ethanol and isopropanol, which are flammable. Guanidine hydrochloride (a component of the Binding Buffer) should be considered harmful and an irritant. Wear gloves and follow standard safety procedures while working with these substances. Refer to the SDS for detailed safety information.

The Maxwell® RSC Cartridges are designed for use with potentially infectious substances. Wear appropriate personal protective equipment (e.g., gloves and safety glasses) when handling infectious substances. Adhere to your institutional guidelines for handling and disposal of all infectious substances used with this system.

Caution: Handle cartridges with care; seal edges may be sharp.

3. Intended Use

The Maxwell® RSC Fecal Microbiome DNA Kit is intended for use in combination with supported Maxwell® Instruments running the Fecal Microbiome DNA method and is for Research Use Only. This kit is intended for use with fecal samples.

4. Sample Preprocessing Protocols

4.A. Before You Begin

Before using the Maxwell® RSC Fecal Microbiome DNA Kit for the first time, the Fecal Microbiome DNA method must be installed on your instrument. Methods are available at:

www.promega.com/resources/software-firmware/maxwell-maxprep/maxwell-rsc-fsc-software-firmware-methods/

The Maxwell® RSC Fecal Microbiome DNA Kit can process 100–300mg of fecal sample per DNA isolation.

The total yield and quality of microbiome DNA from fecal samples depends on the amount of material processed, the amount of genomic DNA in the sample type and the type of fecal material. Sample handling conditions such as storage temperature, number of freeze-thaws and homogenization solutions can also impact yield and quality. Each cartridge supplied in the Maxwell® RSC Fecal Microbiome DNA Kit is designed to purify genomic DNA from 300µl of lysate. Samples are lysed in a larger volume, and only a fraction of the cleared lysate is transferred to the cartridge to avoid sample inhibitors. All reagents needed to lyse samples and purify DNA are included in the kit.

Materials to Be Supplied by the User

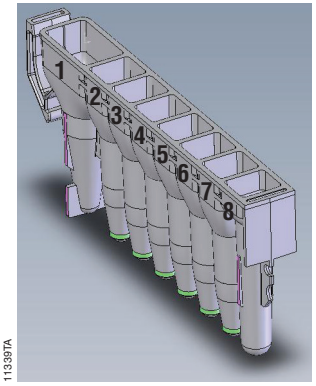
- screw-cap microcentrifuge tubes, 2.0ml
- sterile, aerosol-resistant pipette tips
- heat block suitable for 2.0ml microcentrifuge tubes
- microcentrifuge
- vortex
- **optional:** zirconia or silica beads at 0.1–0.7mm (e.g., Zymo Research Cat.# S6012-50)
- **optional:** horizontal vortex adapter for 1.5/2.0ml tubes

Note: Use either the Sample Lysis protocol, Section 4.B or Sample Lysis with Bead Beating protocol, Section 4.C.

4.B. Sample Lysis

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 tube into a heat block at 95°C for 5 minutes.
4. Remove samples from heat and allow to cool for 2 minutes on the 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 as instructed in Section 5.A.
8. Place lysate tubes into a microcentrifuge and spin at room temperature for 5 minutes at maximum speed (>10,000 × *g*) to pellet any 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 from the surface of the liquid. Also avoid oil on the surface. 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.
10. Proceed to Section 5 for purification on the Maxwell® Instrument.



User Adds to Wells
1. 300µl of Binding Buffer + 300µl of preprocessed sample lysate to well #1 of each cartridge.
3. 20µl of RNase A to well #3 of each cartridge
8. Add a plunger to well #8 of each cartridge

Figure 1. Maxwell® RSC Cartridge.

4.C. Optional: Sample Lysis with Bead Beating

1. Weigh ≤300mg of solid material or measure ≤600µl of a liquid sample and add the sample to a bead beating tube.
2. Add 1ml of Lysis Buffer and 40µl Proteinase K to each sample and cap the tubes tightly.
3 Place tubes in a horizontal tube adapter assembled on a vortex. Bead beat at maximum speed (~3,000rpm) for 30 minutes.

   Note: For high-speed disruptors, typically 3–5 minutes of bead beating with intermittent cooling is advised to prevent tube damage and nucleic acid degradation.
4. Place tube into a heat block at 95°C for 5 minutes.
5. Remove samples from heat and allow to cool for 2 minutes at room temperature.
6. Vortex thoroughly for 1 minute.
7. Incubate samples at 56°C for 5 minutes.
8. During the incubation (Step 7), prepare cartridges as instructed in Section 5.A.
9. Centrifuge lysate tubes in a microcentrifuge for 5 minutes at room temperature and maximum speed (>10,000 × *g*) to pellet any solids.
10. 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 from the surface of the liquid. Also avoid oil on the surface. Transfer of 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.
11. Proceed to Section 5 for purification on the Maxwell® Instrument.

5. Purifying DNA on the Maxwell® Instruments

5.A. Maxwell® RSC Fecal Microbiome DNA Cartridge Preparation

1. Change gloves before handling cartridges, plungers and Elution Tubes. Place the required number of cartridges in the deck tray(s). Place each cartridge in the deck tray with well #1 (the largest well) facing away from the Elution Tube position. 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 cartridges in the instrument.

   Note: Sample or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bactericidal spray or wipe, then water. Do not use bleach on any instrument parts.
2. Place a Maxwell® RSC Plunger into well #8 of each cartridge. Well #8 is the well closest to the Elution Tube position. See Figures 1 and 2.

   Note: Use only the plungers provided in the Maxwell® RSC Fecal Microbiome DNA Kit.

5.A. Maxwell® RSC Fecal Microbiome DNA Cartridge Preparation (continued)

3. Place empty Elution Tubes into the elution position for each cartridge in the deck tray(s). Add 100µl of Elution Buffer to the bottom of each Elution Tube. See Figure 2.

   Notes:

   a. If Elution Buffer is on the side of the tube, the elution may be suboptimal.

   b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.

4. Add 300µl of Binding Buffer to well #1 (the largest well) of each cartridge.

5. Add 20µl of RNase A into well #3 of each cartridge.

6. Add only 300µl of sample lysate processed as instructed in Section 4.B, Step 9 to well #1 of each cartridge. Avoid transferring any solid material from the bottom of the tube or oil from the surface of the liquid.

Figure 2. Setup and configuration in the deck tray(s). Elution Buffer is added to the Elution Tubes as shown. Plungers are in well #8 of the cartridge.

5.B. Maxwell® Instrument Setup and Run

See Table 1 for a list of supported instruments, their catalog and related Technical Manual numbers.

1. Turn on the Maxwell® Instrument and Tablet PC. Log in to the Tablet PC and start the Maxwell® software by double-touching the icon on the desktop. The instrument will power up, proceed through a self-check and home all moving parts.
2. Touch **Start** to begin the process of running a method.
3. Depending on your Maxwell® Instrument model, use one of the following options to select a method:
   a. When running in Portal mode, scan the bar code(s) on the deck tray(s). After data has been returned from the Portal software, touch **Continue** to use the sample tracking information for the deck tray(s) or touch **New** to start a run and enter new sample tracking information.
   b. Touch the **Fecal Microbiome DNA** method, or
   c. Scan or enter the 2D bar code on the kit box to automatically select the appropriate method (Figure 3).
4. If applicable to your Maxwell® Instrument model, verify that the Fecal Microbiome DNA method has been selected, and touch the **Proceed** button. If requested by the software, enter any kit lot and expiration information that has been required by the Administrator.
5. On the 'Cartridge Setup' screen (if shown), touch the cartridge positions to select or deselect any positions to be used for this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.

   Note: When using a 48-position Maxwell® Instrument, touch the **Front** and **Back** buttons to select or deselect cartridge positions on each deck tray.

Figure 3. Kit label indicating the method bar code. Scan the bar code shown in the upper right of this label, to start a purification run.

5.B. Maxwell® Instrument Setup and Run (continued)

6. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that cartridges are loaded on the instrument, preprocessed samples are added to well #1 of the cartridges, uncapped Elution Tubes are present with 100µl of Elution Buffer and plungers are present in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® Instrument platform.

   Inserting the Maxwell® deck tray(s): Hold the deck tray by the sides to avoid dislodging cartridges from the deck tray. Ensure that the deck tray is placed in the Maxwell® Instrument with the elution tubes closest to the door. Angle the back of the deck tray downward and place into the instrument so that the back of the deck tray is against the back of the instrument platform. Press down on the front of the deck tray to firmly seat the deck tray on the instrument platform. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation. Ensure the deck tray is level on the instrument platform and fully seated.

   Note: Check the identifier on the 24-position deck trays to determine whether it should be placed in the front or back of the instrument.

7. Touch the **Start** button to begin the extraction run. The platform will retract, and the door will close.

   Note: When using a 48-position Maxwell® Instrument, if the Vision System has been enabled, the deck trays will be scanned as the door retracts. Any errors in deck tray setup (e.g., plungers not in well #8, elution tubes not present and open) will cause the software to return to the 'Cartridge Setup' screen and problem positions will be marked with an exclamation point in a red circle. Resolve all error states and touch **Start** again to repeat deck tray scanning and begin the extraction run.

   Warning: Pinch point hazard.

   The Maxwell® Instrument will immediately begin the purification run. The screen will display information including the user who started the run, the current method step being performed and the approximate time remaining in the run.

   Notes:

   a. Touching **Abort** will abandon the run.

   b. If the run is abandoned before completion, you will be prompted to check whether plungers are still loaded on the plunger bar. If plungers are present on the plunger bar, perform **Clean Up** when requested. If plungers are not present on the plunger bar, you can choose to skip **Clean Up** when requested. In all cases, the samples will be lost.

8. Follow the on-screen instructions at the end of the method to open the door. Verify that the plungers are located in well #8 of the cartridge at the end of the run. If the plungers are not removed from the plunger bar, follow the instructions in the Technical Manual appropriate to your Maxwell® Instrument (see Table 1) to perform a 'Clean Up' process to attempt to unload the plungers.

9. Remove the deck tray(s) from the instrument. Remove elution tubes containing DNA, and cap the tubes. Centrifuge samples for 2 minutes at full speed, then transfer to a new microcentrifuge tube prior to any downstream analysis. For short-term storage or frequent use of the DNA, store at + 2 to +10°C; for long-term storage, store at −30 to −10°C. Avoid multiple freeze-thaw cycles.

   After the run is complete, the extraction run report is displayed. From the 'Report View' screen, you can print or export this report or both.

   Note: Following the automated purification procedure, the deck tray(s) will be warm but not too hot to touch. To remove the deck tray from the instrument platform, hold onto the sides of the deck tray.

10. Remove the cartridges and plungers from the deck tray(s) and discard as hazardous waste following your institution's recommended guidelines. Do not reuse reagent cartridges, plungers or elution tubes.

    Ensure samples are removed before performing any required UV light treatment to avoid damage to the nucleic acid.

11. Quantitate using a method appropriate for use in your downstream assay. We recommend using DNA-specific fluorescent dye quantitation for determining yield.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Lower than expected yield	Sample is relatively low in DNA content or is degraded. Use more starting material. To prevent degradation, chill samples during preparation.
	Inhibitors present. Avoid transfer of preprocessed sample surface oils and pelleted solids to the cartridge. Repeat spin with cleared lysate to improve separation before transfer to cartridge. Reduce the amount of starting material used per sample. Do not exceed 300mg of sample in the standard protocol lysis.
	Add more of the supernatant to well #1 of the cartridge. Adding up to 500µl of cleared lysate has shown to have little to no inhibition in limited qPCR assays.
	The Maxwell® Instrument was set for the wrong method. Ensure that the Fecal Microbiome DNA Extraction method is selected.
Resin fines are present in the eluate	Resin fines should not affect qPCR. However, if you prefer to remove the fines, briefly centrifuge and transfer the eluate to a clean tube.

7. Related Products

Instruments and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
Maxwell® RSC Plunger Pack	1 each	AS1670
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® FSC Deck Tray	1 each	AS4016
Maxwell® CSC 48 Instrument	1 each	AS8000

Solutions and Buffers

Product	Size	Cat.#
RNase A Solution (4mg/ml)	1ml	A7973
	5ml	A7974
Proteinase K (PK) Solution (20mg/ml)	4ml	MC5005
	16ml	MC5008

8. Summary of Changes

The following changes were made to the 3/22 revision of this document:

1. An optional bead beating protocol has been added, Section 4.C.
2. Kit components have been updated to include one additional tube of RNase A.
3. The cover image was updated.

[a]U.S. Pat. No. 7,329,488 and S. Korean Pat. No. 100483684.