TECHNICAL MANUAL

Maxwell® RSC Buccal Swab DNA Kit

Instructions for Use of Product
AS1640

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

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

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Maxwell® RSC Buccal Swab 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

The Maxwell® RSC Buccal Swab DNA Kit[a] (Cat.# AS1640) is designed to provide an easy method for efficient, automated purification of genomic DNA (gDNA) from buccal swab samples. Maxwell® Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the RSC Buccal Swab DNA Kit can process from one to the maximum sample number in about 40 minutes. The purified DNA can be used directly in a variety of downstream applications, including PCR and agarose gel electrophoresis.

Table 1. Supported Instruments

Instrument	Cat.#	Technical Manual
Maxwell® RSC	AS4500	TM411
Maxwell® RSC 48	AS8500	TM510
Maxwell® FSC	AS4600	TM462
Maxwell® CSC RUO Mode	AS6000	TM573
Maxprep™ Liquid Handler	AS9100, AS9101 AS9200, AS9201	TM509

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1. Description (continued)

The Maxwell® RSC Buccal Swab DNA Kit purifies samples using a novel paramagnetic particle, called the MagnaCel™ particle, which provides a mobile solid phase that optimizes capture, washing and purification of sample gDNA. This particle utilizes cellulose-based binding of nucleic acids and provides a higher bind capacity and cleaner eluate than traditional DNA purification. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind gDNA to the paramagnetic particles in the first well of a prefilled cartridge and mix during processing. This approach to magnetic capture avoids common liquid-handling problems such as clogged tips or partial reagent transfers that result in suboptimal purification processing by other automated systems.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Buccal Swab DNA Kit	**48 preps**	**AS1640**

For Research Use Only. Not for use in diagnostic procedures. Sufficient for 48 automated isolations from 48 buccal swab samples. Cartridges are for single use only. Includes:

- 2 × 1ml Proteinase K (PK) Solution
- 20ml Lysis Buffer
- 48 Maxwell® RSC Cartridges (RSCB)
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes (0.5ml)
- 50 Clearing Columns
- 20ml Elution Buffer

Storage Conditions: Store the kit at 15–30°C.

Available Separately (recommended for sample extraction)

PRODUCT	SIZE	CAT.#
ClickFit Microtube, 1.5ml	**1,000/pack**	**V4741**
RNase A Solution, 4mg/ml	**1ml**	**A7973**

Safety Information: The Maxwell® RSC Cartridges contain ethanol and isopropanol. These substances should be considered flammable, harmful and irritants. Refer to the SDS for more detailed safety information.

Samples used with the Maxwell® RSC Cartridges may contain potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

Caution: Handle cartridges with care; seal edges may be sharp. Bleach reacts with guanidine thiocyanate and should not be added to any sample waste from these cartridges.

3. Before You Begin

Materials to Be Supplied by the User

- microcentrifuge
- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- buccal swabs (e.g., Puritan Medical Products Cat.# 25-806 1PD or 25-806 1PC)
- 1.5–2.0ml tubes for incubation of samples (e.g., ClickFit Microtube, 1.5ml [Cat.# V4741]; recommended to prevent the cap from opening during heating)
- heating block set at 56°C

3.A. Preparation of Buccal Swab Samples

The total yield of genomic DNA from buccal samples depends on the cellular material on the swabs. The amount of cellular material on the swab is dependent on donor, collection technique and donor behavior before collection. See Section 5 for ways to increase cellular collection.

1. Collect samples with a standard buccal swab collection procedure.
2. Assemble a ClickFit Microtube with a Clearing Column for each sample.
3. Cut the head off the applicator stick. Add dried swab head to the Clearing Column and ClickFit Microtube.
4. In a separate tube, mix 300µl of Lysis Buffer + 30µl of Proteinase K (PK) Solution for each sample.
5. Add 330µl of Lysis Buffer/Proteinase K (PK) Solution to each swab head, and close tube over the Clearing Column.

   Note: If using tubes other than the recommended ClickFit Microtube, 1.5ml (Cat.# V4741), the tube may not close.
6. Incubate for 20 minutes at 56°C.

   Note: Some liquid from the Clearing Column may flow through in the ClickFit Microtube after incubation. This is normal.
7. Centrifuge the Clearing Column and ClickFit Microtube with swab for 2 minutes at maximum speed in a microcentrifuge.
8. Discard the Clearing Column and swab head.
9. Add flowthrough liquid to well #1 (the largest well) of the cartridge.

3.B. Maxwell® RSC Buccal Swab DNA Cartridge Preparation

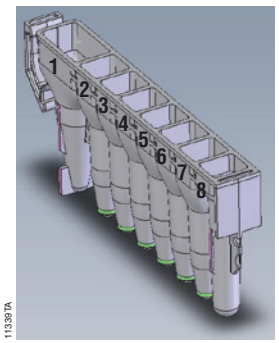
1. Change gloves before handling cartridges, plungers and elution tubes (0.5ml). Place each cartridge in the deck tray(s) with well #1 (the largest well) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
2. Place one plunger into well #8 of each cartridge.

3.B. Maxwell® RSC Buccal Swab DNA Cartridge Preparation (continued)

3. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s). Add 50µl of Elution Buffer to the bottom of each elution tube.

Notes:

1. Optional: If you are concerned about RNA copurification, add 5µl of RNase A Solution (Cat.# A7973) to well #3.
2. If specimen or reagent spills on any part of the deck, wipe up the excess. After the run, follow Section 4, Step 11, to properly clean the deck.



User Adds to Wells
1. Preprocessed Sample
3. Optional: RNase A
8. Plunger

Figure 1. Maxwell® RSC Cartridge.

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

4. Maxwell® Instrument Setup and Run

For detailed information, refer to the Technical Manual specific to your Maxwell® Instrument.

Table 2. Maxwell® Instrument Technical Manuals

Instrument	Technical Manual
Maxwell® RSC	TM411
Maxwell® RSC 48	TM510
Maxwell® FSC	TM462
Maxwell® CSC RUO Mode	TM573

1. Turn on the Maxwell® Instrument and Tablet PC. Sign in to the Tablet PC, and start the Maxwell® Software by double-touching the icon on the desktop. The instrument will 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, press **Continue** to use the sample tracking information for the deck tray(s) or press **New** to start a run and enter new sample tracking information.
   b. Scan or enter the 2D bar code information on the kit box to automatically select the appropriate method.
   c. Touch the **Buccal Swab DNA** method.
4. If applicable to your Maxwell® Instrument model, verify that the Buccal Swab DNA method has been selected, and touch the **Proceed** button. If requested by the software, scan or enter any kit lot information that has been required by the Administrator.
5. On the 'Cartridge Setup' screen (if shown), touch the cartridge positions to select/deselect the positions to be used for this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.

   Note: When using 48-position Maxwell® Instruments, press the **Front** and **Back** buttons to select/deselect cartridge positions on each deck tray.
6. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with Elution Buffer and plungers are 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 24-position Maxwell® deck trays to determine whether they should be placed in the front or back of the instrument.

4. Maxwell® Instrument Setup and Run (continued)

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

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:

1. 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 press the **Start** button again to repeat deck tray scanning and begin the extraction run.
2. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.
3. 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. The samples will be lost.

8. Follow on-screen instructions at the end of the method to open the door. Verify that plungers are located in well #8 of the cartridge at the end of the run. If plungers are not removed from the plunger bar, follow the instructions in the Technical Manual appropriate to your Maxwell® Instrument (see Table 2), 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. After the run has been completed, the extraction run report will be 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. It will not be too hot to touch. To remove the deck tray(s) from the instrument platform, hold onto the sides of the deck tray.

10. Specimen 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. All cartridges and tubes should be removed before cleaning. Do not use bleach on any instrument parts.

11. Remove the cartridges and plungers from the deck tray.

Discard the cartridges and plungers 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.

5. 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 (lower than expected A_{260})	Proteinase K Solution was not added. The lysis and yield are dependent upon complete extraction with Proteinase K.
	Buccal swab was not collected using best practices. • Refrain from eating, drinking, chewing gum or brushing teeth for at least half an hour before sample collection. • Rubbing the cheek more aggressively or with the dominant hand will yield more DNA than rubbing lightly. • Rubbing the cheek for longer (30 seconds) will yield more DNA. • Allow the swab to dry after collection.
	Buccal swab yield is highly donor dependent. A person who sheds cells at a higher rate will yield more DNA than someone who sheds at a lower rate, even when using the same collection technique.
	The Maxwell® Instrument was set for the wrong method. Ensure that the Buccal Swab DNA method is chosen.
Lower than expected yield (lower than expected A_{260})	If you have two swabs from the same donor, it is possible to combine them into one sample by putting both swab heads into a single Clearing Column. To process two swabs together: 1. Add both swab heads into the Clearing Column and ClickFit Microtube microcentrifuge tube assembly. The second swab head may have to be in the opposite orientation for the lid to close. 2. Add 400µl of Lysis Buffer + 30µl of Proteinase K (PK) Solution to the swabs. Pipet slowly enough for the liquid to absorb into the swabs and not overflow. Carefully close the cap. 3. Follow the rest of the protocol as instructed in Section 3.A.
qPCR analysis amplified less DNA than expected	Fluorescence-based DNA quantitation tends to provide a more predictive measure of amplifiable genomic DNA than spectrophotometric quantitation. If a precise measurement of gDNA is required for a downstream assay, we recommend using an intercalating fluorescent dye such as that in the QuantiFluor® dsDNA System (Cat.# E2670).
Instrument unable to pick up plungers	Make sure you are using a Maxwell® RSC kit; the plungers for the Maxwell® RSC reagent kits are specific for the supported Maxwell® Instruments for this kit.

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6. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® FSC Deck Tray	1 each	AS4016
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC 48 Back Deck Tray	1 each	AS8402
Maxwell® RSC Plunger Pack	1 each	AS1670
RNase A Solution, 4mg/ml	1ml	A7973
Proteinase K (PK) Solution, 20mg/ml	4ml	MC5005
ClickFit Microtube, 1.5ml	1,000/pack	V4741

Maxwell® RSC Reagent Kits

Visit **www.promega.com** for a list of available Maxwell® RSC purification kits.

7. Summary of Changes

The following change was made to the 9/19 revision of this document:

1. Updates were made throughout to genericize Maxwell® references for multiple supported instruments.

[a]U.S. Pat. No. 6,855,499, European Pat. Nos. 1368629, 2090655 and 2363476, Japanese Pat. No. 4399164 and other patents.