TECHNICAL MANUAL

Maxwell® RSC Stabilized Saliva DNA Kit

Instructions for Use of Product
AS1630

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

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

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TM480

Maxwell® RSC Stabilized Saliva 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 Stabilized Saliva DNA Kit[a] (Cat.# AS1630) is designed to provide an easy method for efficient, automated purification of genomic DNA (gDNA) from stabilized saliva samples. Maxwell® Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the RSC Stabilized Saliva 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

1. Description (continued)

The Maxwell® RSC Stabilized Saliva 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 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 Stabilized Saliva DNA Kit	**48 preps**	**AS1630**

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

- 48 Maxwell® RSC Cartridge (RSCK)
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Store the kit at ambient temperature (15–30°C).

Available Separately

PRODUCT	SIZE	CAT.#
RNase A Solution, 1ml	**1 tube**	**A7973**
Proteinase K (PK) Solution, 4ml	**1 bottle**	**MC5005**
ClickFit Microtube, 1.5ml	**1,000/pack**	**V4741**

Safety Information: The Maxwell® RSC Cartridges contain ethanol and isopropanol. Ethanol and isopropanol should be considered flammable, harmful and irritants. Refer to the SDS for 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

- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- Optional: Heating block set at 56°C
- Optional: 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)
- Optional: Proteinase K (PK) Solution (Cat.# MC5005) to process stabilized saliva samples that are less than 2 days post-collection
- Optional: RNase A Solution, 4mg/ml (Cat.# A7973)

3.A. Preparation of Stabilized Saliva Samples

The total yield of genomic DNA from stabilized saliva samples depends on the cellular material present in the stabilized saliva. The amount of cellular material in the saliva is dependent on donor, collection technique, and donor behavior before collection. See the troubleshooting section for ways to increase cellular material.

The Maxwell® RSC Stabilized Saliva DNA Kit has been tested with the following stabilized saliva collection devices:

- DNA Genotek Oragene®•DISCOVER DNA Collection Device
- IsoHelix GeneFiX™ Saliva DNA Collection Kit
- Biomatrica DNAgard® Saliva and DNAgard® Saliva HT Collection Device.

Other saliva stabilization products may not work optimally with this purification kit.

1. Collect samples with the appropriate standard saliva collection procedure.

   Note: Storing the collectors containing saliva samples for more than two days after collection may improve extraction. If you want to process samples immediately after collection, consider using a heated preprocessing method (see Section 3.B).

2. Vortex the stabilized saliva until the sample is homogenous (>10 seconds).
3. Add up to 1ml of stabilized saliva sample to well #1 (the largest well) of the cartridge.

3.B. Optional Heated Preprocessing of Stabilized Saliva Samples

If the saliva sample was collected less than two days before purification, you may achieve a modest increase in yield and purity if you first heat the sample (for the Oragene®•DISCOVER DNA Collection Device) or add Proteinase K before heating the sample (for most other saliva collectors).

1. Collect samples with the appropriate standard saliva collection procedure.
2. Vortex the stabilized saliva until the sample is homogenous (>10 seconds).
3. Add up to 1ml of stabilized saliva to a microcentrifuge tube.

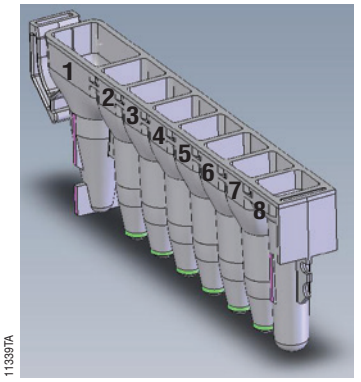
   Note: We recommend the ClickFit Microtube, 1.5ml (Cat.# V4741) because the cap remains securely closed during heating.
4. Add 30µl of Proteinase K to the sample. Vortex briefly.

   Note: For Oragene®•DISCOVER DNA samples, we recommend not adding Proteinase K.
5. Incubate the tube at 56°C for 1 hour.
6. Transfer up to 1ml of the preprocessed sample to well #1 (the largest well) of the cartridge.

3.C. Maxwell® RSC Stabilized Saliva DNA Cartridge Preparation

1. Change gloves before handling Cartridges, Plungers and Elution Tubes. Place the cartridges to be used in the deck tray. Place each cartridge in the deck tray(s) with well #1 (the largest well in the cartridge) 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. Optional: If RNA co-purification is a concern, 5µl of RNase A Solution, 4mg/ml (Cat.# A7973) can be added to well #3.
4. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s). Add 150µl of Elution Buffer to the bottom of each elution tube.

   Note: Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bacteriocidal spray or wipe, then water. All cartridges and tubes should be removed before cleaning. Do not use bleach on any instrument parts.



User Adds to Wells

1. Preprocessed Stabilized Saliva
3. Optional: RNase A
8. RSC 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. The plunger is 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 **Stabilized Saliva DNA** method.
4. If applicable to your Maxwell® Instrument model, verify that the Stabilized Saliva DNA method has been selected, and press 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 press 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 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 150µl 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 24-position Maxwell® deck trays to determine whether they 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.

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; 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 and the samples 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, you should perform **Clean Up** when requested. If plungers are not present on the plunger bar, you can choose to skip **Clean Up** when requested.

9. 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 unload the plungers.

10. 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 and/or export this report.

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

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

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})	If using Norgen Biotek Saliva DNA Collection and Preservation Device, a stronger lysis buffer is required for optimal yield and purity. For this particular sample type, we recommend using the Maxwell® RSC Blood DNA kit (Cat.# AS1400). Follow the Maxwell® RSC Blood DNA protocol, using 300µl of Norgen's Saliva DNA sample in place of 300µl of blood.
	Saliva 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 tongue around the mouth or moving saliva past the cheek before spitting can increase yield. • Ensure that the saliva volume—not including foam or bubbles—reaches the fill mark.
	Vortex the sample well before processing. If the tube is too full to form a vortex, vigorously invert the tube until the settled material on the bottom is homogenously mixed into solution.
	The Maxwell® Instrument was set for the wrong method. Ensure that the Stabilized Saliva DNA method is chosen.
	The elution volume added to the elution tubes can be reduced down to 50µl in order to generate a more concentrated sample.
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 the use of an intercalating fluorescent dye such as QuantiFluor® dsDNA (Cat.# E2670).
Instrument unable to pick up plungers	Make sure you are using an RSC-specific kit; the plungers for the Maxwell® RSC reagent kits are specific for the supported Maxwell® Instruments for this kit.

6. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® FSC Deck Tray	1 each	AS4016
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
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; and Japanese Pat. No. 4399164.