TECHNICAL MANUAL

Maxwell® RSC Tissue DNA Kit

Instructions for Use of Product
AS1610

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

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

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Maxwell® RSC Tissue 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 Tissue DNA Kit[a] (Cat.# AS1610) is used with the Maxwell® Instruments specified below to provide a simple method for efficient, automated purification of genomic DNA (gDNA) from tissue samples. Maxwell® Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the RSC Tissue DNA Kit can process from one to the maximum sample number in about 45 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 Tissue DNA Kit purifies samples using a silica-based paramagnetic particle, called the MagneSil® particle, which provides a mobile solid phase that optimizes capture, washing and purification of sample gDNA. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind gDNA to the paramagnetic particle 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 Tissue DNA Kit	**48 preps**	**AS1610**

For Research Use Only. Not for use in diagnostic procedures. Sufficient for 48 automated isolations from up to 50mg tissue samples. Cartridges are for single use only. Includes:

- 48 Maxwell® RSC Cartridge (RSCI)
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 10ml 1X TE Buffer (pH 7.5)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® RSC Tissue DNA Kit at 15–30°C.

Safety Information: The Maxwell® RSC Cartridges contain ethanol, isopropanol and guanidine thiocyanate. Ethanol and isopropanol should be considered flammable, harmful and irritants. Guanidine thiocyanate should be considered toxic, harmful and an irritant. 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

- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- Potter-Elvehjem teflon-coated pestle or Disposable Pellet Pestles with Tube (e.g., Fisher Cat.# 12-141-368)

  Note: Some tissues will require more vigorous disruption techniques than a disposable pestle will allow. For these tissues, a Potter-Elvehjem homogenizer is strongly recommended. Tissue that is not homogeneously disrupted may result in poor purification performance, and tissue may carry through into the elution tube.
- (Optional) RNase A Solution, 4mg/ml (Cat.# A7973)

! Disrupting tissues with bead beaters or wand homogenizers will cause DNA shearing prior to purification.

4. Sample Preparation

4.A. Preparation of Tissue Samples

The total yield of genomic DNA from tissue samples depends on the tissue type and mass of tissue to be processed. Each Maxwell® RSC Cartridge supplied in the Maxwell® RSC Tissue DNA Kit is designed to purify genomic DNA from up to 50mg of tissue sample. Ensure that the tissue is well homogenized before processing. We recommend homogenizing tissue samples with a Potter-Elvehjem Teflon-coated pestle. Other methods of homogenization (wand homogenizer, bead beating) will result in shearing of genomic DNA.

Instructions for homogenizing tissue samples with a pestle and tube:

1. Weigh out up to 50mg of tissue sample and place in tube.
2. Add 80µl of TE buffer to the sample in the tube.
3. (Optional) Add 20µl of RNase A Solution (4mg/ml) (Cat.# A7973) to the sample.
4. Using the pestle, thoroughly disrupt and homogenize the tissue sample.

! Tissue that is not homogeneously disrupted may result in poor purification performance, and tissue may carry through into the elution tube.

5. Repeat Steps 1–4 for each tissue sample to be processed.

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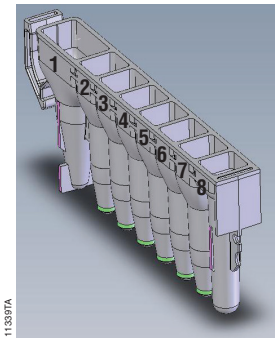
4.B. Maxwell® RSC Tissue DNA Cartridge Preparation

1. Change gloves before handling Maxwell® RSC Cartridges, Plungers and Elution Tubes. Place the cartridges to be used in the deck tray. Place each cartridge 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. 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. Transfer each homogenized tissue sample to well #1 (the largest well) of each cartridge. Mix the homogenized tissue sample into the lysis buffer by pipetting 10 times. Change pipette tips between samples.

   Note: Tip-mix the homogenized tissue sample into the lysis buffer by pipetting at least 10 times to ensure all sample has been transferred.
3. Place one plunger into well #8 of each cartridge.
4. Place an empty elution tube into the elution tube position for each cartridge in the deck tray. Add 100µl of Elution Buffer to the bottom of each elution tube. The starting volume of Elution Buffer will not be the same as the eluted volume after running the method.

   Notes:

   1. 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, and then water. Do not use bleach on any instrument parts.
   2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instruments.
   3. Typically, the final eluted volume will be approximately 30–50µl less than the starting volume.
   4. Certain tissue types (e.g., brain) may result in significant amounts of resin carryover on the sides of the Elution Tube. This outcome is expected and should not affect downstream applications. We recommend transferring the contents to a clean tube for storage if downstream analysis is not performed immediately.



User Adds to Wells

1. Homogenized tissue sample (up to 50mg tissue)
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. Plungers are in well #8 of the cartridge.

5. 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. Log in to the Tablet PC, and start the Maxwell® software on the Tablet PC. 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 **Tissue DNA** method.
4. If applicable to your Maxwell® Instrument model, verify that the Tissue DNA method is selected, and touch the **Proceed** button. If requested by the software, scan or 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/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 deck tray, uncapped elution trubes are present with 100µl of 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.

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. Touch the exclamation point for a description of the error and resolve all error states. Touch the **Start** button again to repeat deck tray scanning and begin the extraction run.
   2. Pressing 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.

5. Maxwell® Instrument Setup and Run (continued)

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. After purification, the elution tubes may have resin that adheres to the side of the tube. This is normal and will not affect downstream assay performance.

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(s) 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.

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 A_{260} (lower than expected yield)	Tissue sample has been stored unfrozen for an extended period of time or has undergone multiple freeze-thaw cycles. Avoid these storage conditions.
	Sample type contains low amount of DNA per mass, or a small mass of tissue sample was used. The yield of genomic DNA from tissue samples depends on the tissue type and the mass of tissue processed.
Lower than expected purity ratios (low A_{260}/A_{280} or A_{260}/A_{230} ratios)	Tissue sample has been stored unfrozen for an extended period of time or has undergone multiple freeze thaw cycles. Avoid these storage conditions.
RNA contamination in DNA eluates	In some cases, RNA can be copurified with the genomic DNA. To remove copurified RNA, perform the optional addition of RNase A to the sample during homogenization (Section 4.A).
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.
Resin carryover on the sides of the Elution Tubes	Certain tissue types (e.g., brain) are likely to result in carryover of resin on the side of the elution tube. This will not affect the performance of eluates in downstream reactions. Transfer the clear eluate to a fresh storage tube before further use.
Pieces of tissue coming through in wells 2–7 of the cartridge or in the Elution Tubes	Tissue was not fully disrupted before placing into the cartridge. Be sure to fully homogenize tissue prior to putting into the cartridge.

7. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® FSC Instrument	1 each	AS4600
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 Deck Tray	1 each	AS4016
RNase A Solution, 4mg/ml	1ml	A7973

Maxwell® RSC Reagent Kits

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

8. Summary of Changes

The following change was made to the 7/20 revision of this document:

1. Product names for Cat.# AS8401 and AS8402 were updated.

[a]U.S. Pat. Nos. 6,027,945 and 6,368,800 and other patents pending.