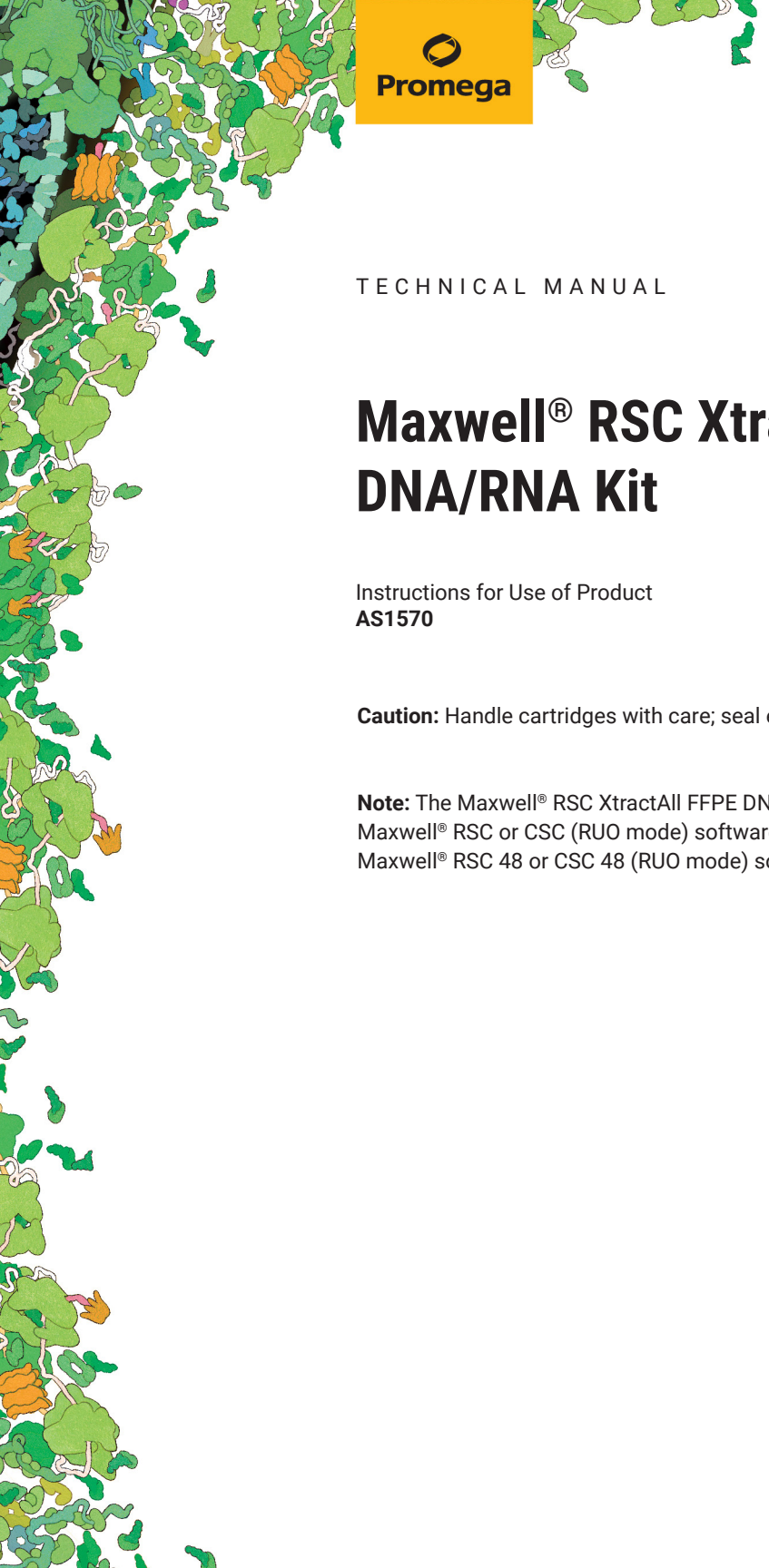

TECHNICAL MANUAL

Maxwell® RSC XtractAll FFPE DNA/RNA Kit

Instructions for Use of Product
AS1570

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

Note: The Maxwell® RSC XtractAll FFPE DNA/RNA Kit is only compatible with Maxwell® RSC or CSC (RUO mode) software version 4.0.0 or greater or Maxwell® RSC 48 or CSC 48 (RUO mode) software version 4.1.1 or greater.

Revised 7/25
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Maxwell® RSC XtractAll FFPE DNA/RNA Kit

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

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1. Description

The Maxwell® RSC XtractAll FFPE DNA/RNA Kit is used in combination with the Maxwell® Instruments specified in Table 1 to provide an easy method for efficient, automated extraction of DNA, RNA, total nucleic acid, or sequential DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples. The Maxwell® RSC Instruments are designed for use with predispensed reagent cartridges and additional reagents supplied in the kit with preprogrammed extraction methods, thereby maximizing simplicity and convenience. The Maxwell® RSC Instruments can process from one to the maximum number of samples allowed in an efficient manner with DNA, RNA and total nucleic acid (TNA) samples being extracted in approximately 30 minutes and sequential DNA and RNA extractions in less than 1 hour. Extracted DNA, RNA or total nucleic acid can be used directly in downstream amplification-based assays.

Table 1. Supported Instruments.

Instrument	Cat.#	Technical Manual	Maximum Sample Number
Maxwell® RSC	AS4500	TM411	16
Maxwell® RSC 48	AS8500	TM510	48
Maxwell® FSC	AS4600	TM462	16
Maxwell® CSC RUO Mode	AS6000	TM573	16
Maxwell® CSC 48 RUO Mode	AS8000	TM628	48

Principle of the Method: The Maxwell® RSC XtractAll FFPE DNA/RNA Kit extracts nucleic acid using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and extraction of DNA, RNA or total nucleic acid. Optionally, DNA and RNA can be sequentially extracted from the same FFPE tissue sample without the need for splitting the lysate. The Maxwell® RSC Instruments are magnetic particle-handling instruments. This system allows efficient binding of nucleic acid to the paramagnetic particles in the first well of a prefilled cartridge and moves the sample through the wells of the cartridge. This approach to magnetic capture avoids common problems, such as clogged tips or partial reagent transfers, which result in suboptimal extraction processing by other commonly used automated systems.

Sample Considerations: Nucleic acid extraction from FFPE tissue samples can be challenging due to tissue characteristics such as fibrosity, lipid composition, nuclease levels and the cell number available in the tissue section. In addition, variability in how the tissue is handled prior to and during fixation, including the duration for which the tissue is exposed to formalin during the tissue fixation process, greatly influences the degree of crosslinking and nucleic acid fragmentation in the FFPE tissue. All these attributes can influence the nucleic acid quality and amount of amplifiable nucleic acid that can be extracted from FFPE tissue sections. During development, the Maxwell® RSC XtractAll FFPE DNA/RNA Kit was evaluated with a variety of human FFPE tissue types to extract the available amplifiable DNA, RNA or total nucleic acid.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC XtractAll FFPE DNA/RNA Kit	**48 preps**	**AS1570**

For Research Use Only. Not for use in diagnostic procedures. Sufficient for 48 automated isolations from FFPE tissue samples. The Maxwell® RSC Cartridges are for single use only. Includes:

- 35ml Mineral Oil
- 20ml Lysis Buffer
- 2 × 1ml Proteinase K
- 2 × 100µl Blue Dye
- 2 × 1ml $MnCl_2$, 0.09M
- 3 vials DNase I (lyophilized)
- 48 Maxwell® RSC Cartridges (RSCR)
- 1 Maxwell® RSC Plunger Pack (48 plungers)
- 2 × 50 Elution Tubes (0.5ml)
- 25ml Nuclease-Free Water

Storage Conditions: Store the Maxwell® RSC XtractAll FFPE DNA/RNA Kit at ambient temperature (+15°C to +30°C). Store rehydrated DNase I at –30°C to –10°C. Avoid more than ten freeze-thaw cycles.

Safety Information: The cartridges contain ethanol and isopropanol. These substances should be considered flammable, harmful and irritants.

The Maxwell® RSC XtractAll FFPE DNA/RNA Kit components are designed to be used 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 the handling and disposal of all infectious substances used with this system.

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

Additional Information: For additional safety information, see the Safety Data Sheet, available at: **www.promega.com**

3. Before You Begin

Materials to Be Supplied By the User

- microcentrifuge
- benchtop vortex mixer
- pipettors and pipette tips for sample preprocessing and transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for incubation of samples (e.g., Microtubes, 1.5ml; Cat.# V1231)
- heat blocks set at 56°C and at 90°C
- FFPE tissue samples (**Note:** Store samples at room temperature [15–30°C].)
- isopropanol, ≥99.5% Molecular Biology Grade (for RNA, TNA and DNA/RNA Sequential workflows)
- razor blades (**Note:** Use caution when scraping samples from the slide with a razor blade.)

As necessary, reconstitute a lyophilized vial of DNase I with 275µl of Nuclease-Free Water and 15µl of Blue Dye. Invert the vial to recover any DNase I from the underside of the cap and swirl gently to mix; **do not** vortex. Store reconstituted DNase I at –30°C to –10°C.

3.A. Preparing FFPE Tissue Samples

Maintain an RNase-free environment during processing. Always use RNase-free and aerosol-resistant pipette tips. Change gloves frequently to reduce the chance of RNase contamination. See Section 9, Creating a Ribonuclease Free Environment, for details.

During development, optimal kit performance was obtained with up to 20µm total thickness of FFPE tissue sections. Multiple sections can be combined in one sample tube for extraction with the maximum thickness of combined sections ≤80µm. Sections thicker than 20µm will affect the Proteinase K digestion and result in low yields (see Section 8). The user should optimize the number of sections and section thickness for compatibility with the laboratory downstream analysis.

During development, the Maxwell® RSC XtractAll FFPE DNA/RNA Kit was evaluated with breast, liver and uterine FFPE tissue samples and found to provide acceptable performance. A wider range of FFPE tissue types may be compatible with the extraction chemistry but should be evaluated by the laboratory for extraction performance and compatibility with downstream assays.

Preprocessing of FFPE Tissue Section Samples

1. Place the FFPE tissue section(s) into a 1.5ml microcentrifuge tube. If you are using slide-mounted FFPE tissue sections, scrape the section(s) off the slide using a clean razor blade.
2. Add 500µl of Mineral Oil to the sample tubes. Vortex for 10 seconds.
3. Heat the samples at 90°C for 5 minutes. Place the samples at room temperature while the master mix is prepared as described in Step 4.

4. Immediately before use, prepare a master mix of the Lysis Buffer, Proteinase K and Blue Dye as shown below:

Reagent	Amount/Reaction	Reactions (Number to be run + 2)	Total
Lysis Buffer	224µl	n + 2	224µl × (n + 2)
Proteinase K	25µl	n + 2	25µl × (n + 2)
Blue Dye	1µl	n + 2	1µl × (n + 2)

5. Add 250µl of master mix to each sample tube, and vortex for 5 seconds.

   Note: Do not store any remaining unused master mix.

6. Centrifuge sample tubes at 10,000 × *g* for 20 seconds to separate the layers. If a pellet is present in the aqueous layer (lower, blue layer), gently mix with a pipette tip to disperse the pellet. Avoid disturbing the mineral oil and aqueous layers in the tube as much as possible.

7. Transfer the sample tubes to a 56°C heat block and incubate for 15 minutes.

8. Transfer the sample tubes to a 90°C heat block and incubate for 1 hour.

9. Proceed to Section 4 for cartridge preparation.

4. Maxwell® RSC XtractAll FFPE DNA/RNA Cartridge Preparation

4.A. Preparing the Maxwell® RSC XtractAll FFPE DNA/RNA Cartridge

1. Change gloves before handling the Maxwell® RSC Cartridges (RSCR), RSC Plungers and Elution Tubes. The cartridges are set up in the deck tray(s) outside of the instrument, and the deck tray(s) containing the cartridges and samples are transferred to the instrument for extraction. Place each cartridge in the deck tray with well #1 (the largest well in the cartridge) farthest away from the Elution Tubes (Figure 1). Press down on the cartridge to snap it into position. Ensure both cartridge ends are fully seated in the deck tray. Carefully peel back the seal to remove the entire seal from the top of the cartridge. Ensure that all sealing tape is removed from the cartridge.

   Caution: Handle cartridges with care. Seal edges may be sharp.

2. Place one plunger into well #8 of each cartridge.

3. Place an empty Elution Tube into the Elution Tube position for each cartridge in the deck tray(s).

   Note: Only use the elution tubes provided in the Maxwell® RSC XtractAll FFPE DNA/RNA Kit. Other elution tubes may not be compatible with the Maxwell® RSC Instrument and could affect extraction performance.

4. Add 30–100µl of Nuclease-Free Water to the bottom of each Elution Tube. Keep the elution tubes open during the extraction (Figure 1).

   Note: Use only the Nuclease-Free Water provided in the Maxwell® RSC XtractAll FFPE DNA/RNA Kit. Using other elution buffers may affect extraction performance or downstream use.

5. Proceed to the appropriate section listed below for instructions specific to each extraction workflow.

Extraction Type	Section
DNA	4.B
RNA	4.C
Total nucleic acid (TNA)	4.D
Sequential DNA and RNA	4.E

Deck Tray Preparation Notes

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.

Figure 1. Setup and configuration of the deck tray. Nuclease-Free Water is added to the Elution Tubes as indicated.

4.B. DNA Extraction Protocol

1. After the end of the 1-hour incubation (Section 3.A), transfer the blue aqueous phase to well #1 of the Maxwell® RSC Cartridge (RSCR). Use a new pipette tip for each sample to avoid cross contamination.

   Notes:

   a. If any undigested material remains at the end of incubation, centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.

   b. Transfer the blue aqueous phase to the cartridge and extract within 30 minutes after completing incubation.

2. Touch **Start** on the 'Home' screen.

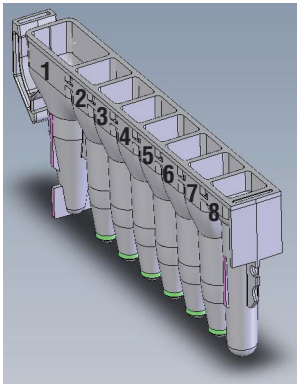
3. On the 'Methods' screen, select a method using one of the two options below:

   a. Select the XtractAll FFPE DNA method and touch the **Proceed** button.

   b. Use a bar code reader to scan the bar code on the kit box to display the four available methods. Select the XtractAll FFPE DNA method and touch the **Proceed** button.

4. Place the deck tray in the Maxwell® instrument, enter cartridge and sample identifier information on the 'Cartridge Setup' screen, confirm the Extraction Checklist items have been performed, and touch the **Start** button to begin the extraction run.

   Note: For detailed instrument setup instructions, refer to Section 5.



User Adds to Wells:

1. Preprocessed samples
8. RSC Plunger

Figure 2. Maxwell® RSC Cartridge. The preprocessed FFPE tissue sample is added to well #1, and a plunger is added to well #8.

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4.C. RNA Extraction Protocol

1. After the end of the 1-hour incubation (Section 3.A), transfer the blue aqueous phase to well #1 of the Maxwell® RSC Cartridge (RSCR). Use a new pipette tip for each sample to avoid cross contamination.

   Notes:

   a. If any undigested material remains at the end of incubation, centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.

   b. Transfer the blue aqueous sample to the cartridge and extract within 30 minutes after completing incubation.

2. Immediately before use, prepare a cocktail of $MnCl_2$ and DNase I as shown below:

Reagent	Amount/Reaction	Reactions (Number to be run + 2)	Total
$MnCl_2$, 0.09M	17µl	n + 2	17µl × (n + 2)
DNase I (with Blue Dye)[1]	10µl	n + 2	10µl × (n + 2)

[1]Store remaining reconstituted DNase I with Blue Dye at –30°C to –10°C.

3. Add 27µl of DNase cocktail to well #7 of each cartridge.

   Note: Do not store remaining unused DNase cocktail.

4. Add 500µl of 100% isopropanol to well #1.

   Note: For purifying small RNAs, add 1,200µl of 100% isopropanol to well #1 and 375µl of 100% isopropanol to well #7.

5. Touch **Start** on the 'Home' screen.

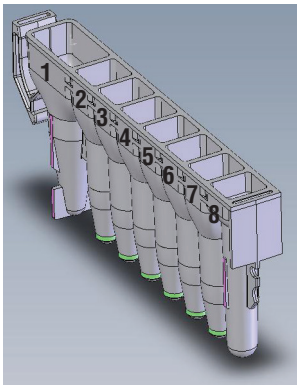
6. On the 'Methods' screen, select a method using one of the two options below:

   a. Select the XtractAll FFPE RNA method and touch the **Proceed** button.

   b. Use a bar code reader to scan the bar code on the kit box to display the four available methods. Select the XtractAll FFPE RNA method and touch the **Proceed** button.

7. Place the deck tray in the Maxwell® instrument, enter cartridge and sample identifier information on the 'Cartridge Setup' screen, confirm the Extraction Checklist items have been performed, and touch the **Start** button to begin the extraction run.

   Note: For detailed instrument setup instructions, refer to Section 5.



User Adds to Wells:

1. Preprocessed samples and 500µl of 100% isopropanol
7. 27µl of DNase cocktail
8. RSC Plunger

Figure 3. Maxwell® RSC Cartridge. The preprocessed FFPE tissue sample and isopropanol are added to well #1, DNase cocktail to well #7, and a plunger is added to well #8.

4.D. Protocol for Extracting Total Nucleic Acid

1. After the end of the 1-hour incubation (Section 3.A), transfer the blue aqueous phase to well #1 of the Maxwell® RSC Cartridge (RSCR). Use a new pipette tip for each sample to avoid cross contamination.

   Notes:

   a. If any undigested material remains at the end of incubation, centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.

   b. Transfer the blue aqueous phase to the cartridge and extract within 30 minutes after completing incubation.

2. Add 500µl of 100% isopropanol to well #1.

   Note: For purifying small RNAs, add 1,200µl of 100% isopropanol to well #1 and 375µl of 100% isopropanol to well #7.

3. Touch **Start** on the 'Home' screen.

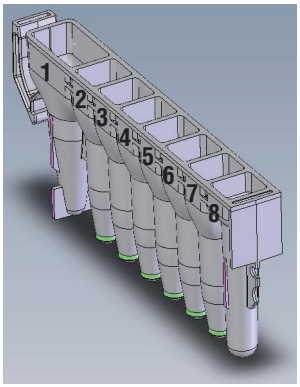
4. On the 'Methods' screen, select a method using one of the two options below:

   a. Select the XtractAll FFPE Total Nucleic Acid method and touch the **Proceed** button.

   b. Use a bar code reader to scan the bar code on the kit box to display the four available methods. Select the XtractAll FFPE Total Nucleic Acid method and touch the **Proceed** button.

5. Place the deck tray in the Maxwell® instrument, enter cartridge and sample identifier information on the 'Cartridge Setup' screen, confirm the Extraction Checklist items have been performed, and touch the **Start** button to begin the extraction run.

   Note: For detailed instrument setup instructions, refer to Section 5.



User Adds to Wells:

1. Preprocessed samples and 500µl of 100% isopropanol
8. RSC Plunger

Figure 4. Maxwell® RSC Cartridge. The preprocessed FFPE tissue sample and isopropanol are added to well #1, and a plunger is added to well #8.

4.E. Protocol for Sequentially Extracting DNA and RNA

When selecting the sequential extraction method, the Maxwell® software will proceed through two distinct extraction runs in succession with the user adding reagents to the cartridges between these runs. For the Maxwell® RSC XtractAll FFPE DNA/RNA Kit, the first method extracts DNA from the lysed FFPE sample into the first elution tube while the second method extracts RNA from the same sample into a second elution tube using the same cartridge and plunger. Below are the instructions for preparing cartridges for each of these extraction runs.

Note: If the Maxwell® Instrument Sanitization setting for "Sanitize after extraction" is turned ON, the UV treatment will automatically be performed after the XtractAll FFPE DNA (Sequential) extraction method has completed and the "Close Door" option has been selected. It is important to remove the Maxwell® Deck Tray(s) with the cartridges and samples from the instrument before beginning the UV treatment. The "Sanitize after extraction" setting may be turned ON or OFF in the Maxwell® Instrument Sanitization settings. Please refer to the Technical Manual for your Maxwell® Instrument for details on managing this setting (see Table 1).

Run 1: DNA Extraction

1. After the end of the 1-hour incubation (Section 3.A), transfer blue, aqueous phase containing the DNA and RNA to well #1 of the Maxwell® FFPE Cartridge (RSCR).

   Notes:

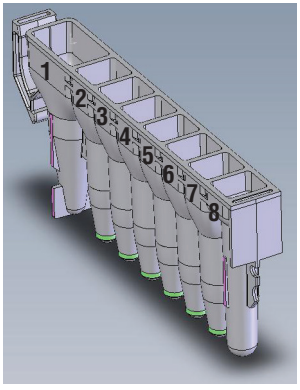
   a. If any undigested material remains at the end of incubation, centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.

   b. Transfer the sample to the cartridge and extract within 30 minutes after completing incubation.

2. Touch **Start** on the 'Home' screen.

3. On the 'Methods' screen, select a method using one of the two options below:
   a. Select the XtractAll FFPE Sequential method and touch the **Proceed** button.
   b. Use a bar code reader to scan the bar code on the kit box to display the four available methods. Select the XtractAll FFPE Sequential method and touch the **Proceed** button.
4. Place the deck tray in the Maxwell® instrument, enter cartridge and sample identifier information on the 'Cartridge Setup' screen, confirm the Extraction Checklist items have been performed, and touch the **Start** button to start the extraction run.

   Note: For detailed instrument setup instructions, refer to Section 5.



User Adds to Wells:

1. Preprocessed samples
8. RSC Plunger

Figure 5. Maxwell® RSC Cartridge. The preprocessed FFPE sample is added to well #1, and a plunger is added to well #8.

Between Run Instructions

Between the first and second extraction runs for the sequential method, perform the following steps:

5. Follow on-screen instructions at the end of the method to open the door. Verify that the plungers are located in well #8 of the cartridges 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 1) to perform a Clean Up process to unload the plungers. To prepare for the Sequential RNA extraction run, a new cartridge setup screen will show.
6. Cap and remove the Elution Tubes containing DNA immediately following the run to prevent eluate evaporation.
7. At the end of the Sequential DNA extraction run, the resin is deposited in well #2 to prepare for the Sequential RNA extraction run.

   Notes:
   a. Do not remove or dispose of cartridges or plungers from the deck tray. They will be reused for the Sequential RNA extraction.
   b. Proceed to Sequential RNA extraction within 2 hours after completing the Sequential DNA extraction.
8. A cartridge setup screen will be shown, indicating the sample positions and identifiers entered before the first DNA extraction run. If necessary, this information can be edited to reflect any changes to the cartridges being processed by touching the **Enable Editing** button.

4.E. Protocol for Sequentially Extracting DNA and RNA (continued)

9. Touch the **Proceed** button to bring up the 'Extraction Checklist' screen.

Run 2: RNA Extraction

10. Place an empty Elution Tube into the Elution Tube position for each cartridge in the deck tray(s).

    Note: Use only the Elution Tubes provided in the Maxwell® RSC XtractAll FFPE DNA/RNA Kit. Other elution tubes may not be compatible with the Maxwell® RSC Instrument and may affect RNA extraction performance.

11. Add 30–100µl of Nuclease-Free Water to the bottom of each Elution Tube. The Elution Tubes must remain open during the RNA extraction.

    Note: Use only the Nuclease-Free Water provided in the Maxwell® RSC XtractAll FFPE DNA/RNA Kit. Using other elution buffers may affect RNA extraction performance or downstream use.

12. Immediately before use, prepare a cocktail of $MnCl_2$ and DNase I as shown below:

Reagent	Amount/Reaction	Reactions (Number to be run + 2)	Total
$MnCl_2$, 0.09M	17µl	n + 2	17µl × (n + 2)
DNase I[1] (with Blue Dye)	10µl	n + 2	10µl × (n + 2)

[1]Store remaining reconstituted DNase I with Blue Dye at –30°C. to –10°C.

13. Add 27µl of DNase cocktail to well #7 of each cartridge.

    Note: Do not store excess unused DNase cocktail.

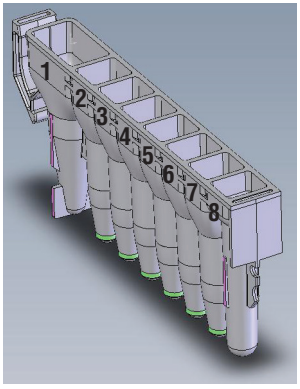
14. Add 500µl of 100% isopropanol to well #1.

    Note: For purifying small RNAs, add 1,200µl of 100% isopropanol to well #1 and 375µl of 100% isopropanol to well #7.

15. Place the deck tray in the Maxwell® instrument, confirm the Extraction Checklist items have been performed and touch the **Start** button to start the second (RNA) extraction run.

Sequential RNA Extraction Cartridge Preparation Notes

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.



User Adds to Wells:

1. 500µl of 100% isopropanol (Add to existing sample in well #1)
7. 27µl of DNase cocktail
8. RSC Plunger (same plunger used during Sequential DNA extraction)

Figure 6. Maxwell® RSC Cartridge. Isopropanol is added to the existing sample in well #1, DNase cocktail to well #7, and the same plunger used in the sequential DNA extraction run should be present in well #8.

Figure 7. Setup and configuration of the deck tray. Nuclease-Free Water is added to the Elution Tubes as indicated.

5. Maxwell® Instrument Setup and Run

For detailed information, refer to the Technical Manual specific to your Maxwell® Instrument. See Table 1.

Note: The Maxwell® RSC XtractAll FFPE DNA/RNA Kit is only compatible with Maxwell® RSC or CSC (RUO mode) software version 4.0.0 or greater or Maxwell® RSC 48 or CSC 48 (RUO mode) software version 4.1.1 or greater.

1. Turn on the Maxwell® Instrument and Tablet PC. Log into 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** on the 'Home' screen.
3. On the 'Methods' screen, select a method using one of the two options below:
   a. Select the XtractAll FFPE method that corresponds to the workflow you are running.
   b. Use a bar code reader to scan the 2D bar code on the kit box to bring up the four available methods (Figure 8). Select the XtractAll FFPE method that corresponds to the workflow you are running.

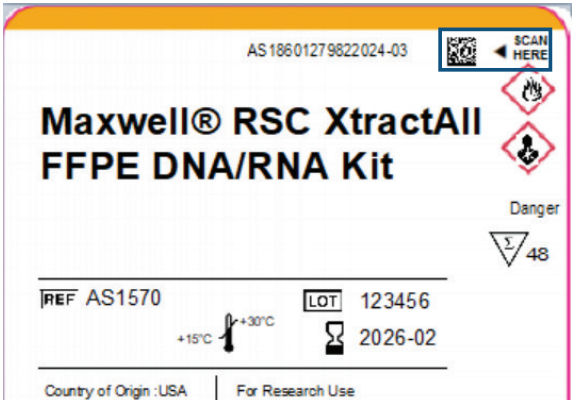


Figure 8. Kit label indicating the bar code to scan. The bar code to scan for starting a purification run is shown in the blue box, on the upper right of the kit label.

4. Verify that the correct extraction method has been selected and touch the **Proceed** button.
5. On the 'Cartridge Setup' screen, 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 the Maxwell® RSC 48 Instrument, touch the **Front** or **Back** button to select or deselect cartridge positions on each deck tray.

6. After the door has opened, confirm that all extraction checklist items have been performed. Verify that preprocessed samples were added to well #1 of the cartridges, isopropanol was added to well #1 of the cartridge (for RNA and TNA workflows only), DNase cocktail was added to well #7 of the cartridge (for RNA workflow only), cartridges are loaded on the instrument, uncapped elution tubes are present with Nuclease-Free Water and plungers are in well #8. Transfer the deck tray containing the prepared cartridges to the Maxwell® instrument platform.

   Inserting the Maxwell® deck tray: 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. Confirm all the indicated preprocessing has been performed, and touch **Start** to close the instrument door and start processing.

   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. 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.

Warning: Pinch point hazard.

8. The Maxwell® Instrument will immediately begin the extraction run. The screen will display the steps performed and the approximate time remaining in the run.

   Notes:

   a. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.

   b. If the run is abandoned before completion, you may 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. The samples will be lost.

9. When the run (DNA, RNA, TNA workflows) or both sequential runs are complete, the user interface will display a message that the method has completed.

End of Run

10. Follow 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 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.

5. Maxwell® Instrument Setup and Run (continued)

11. Cap and remove the Elution Tubes containing your nucleic acid immediately following the run to prevent evaporation of the eluates. Remove the Maxwell® deck tray(s) from the instrument.

    Note: To remove the deck tray from the instrument platform, hold the deck tray by its sides. Ensure the samples are removed from the instrument before running a UV sanitization protocol to avoid damage to the extracted nucleic acid. DNA samples can be stored for up to one week at 4°C and up to one month at –20°C. RNA and TNA samples may be stored overnight at –30°C to –10°C, or at lower than –60°C for longer-term storage.

12. Remove the cartridges and plungers from the Maxwell® deck tray(s) and discard as hazardous waste according the procedures for your institution. Cartridges, plungers and elution tubes are intended for single use. Do not reuse Maxwell® RSC Cartridges, RSC Plungers or Elution Tubes.

6. Workflow Efficiencies

The sequential workflow of the Maxwell® RSC XtractAll FFPE DNA/RNA kit combines the DNA only extraction method and the RNA only extraction method into a single protocol. Because of this, you can run multiple extractions within the same sequential workflow. This means you can sequentially extract DNA from samples before extracting RNA from the same samples. The sequential workflow is uniquely designed to extract DNA and RNA into separate elution tubes while conserving sample tracking information, but the sample tracking is flexible enough to accept modifications between the two runs.

	Workflows in Sequential Method		
Step	**Sequential DNA and RNA**	**DNA Only**	**RNA Only**
Add samples, cartridges and sample tracking information	Add	Add	
First extraction (DNA) of sequential method	X	X	
Remove DNA eluates from instrument	X	X	
Amend samples, cartridges and sample tracking information	X	Remove	Add
Second extraction (RNA) of sequential method	X		X
Remove RNA eluates from instrument	X		X

7. Post-Extraction Instructions

Determine that the extracted nucleic acid yield and purity meets the input requirements for the desired downstream assay. Kit performance was evaluated based on the extraction of amplifiable DNA, RNA and TNA, and fluorescent dye binding. Other quantification methods, including absorbance, may not correlate with amplification or fluorescence. Absorbance readings for FFPE samples may overestimate yield; we recommend using more specific methods for determining yield.

8. Troubleshooting

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

Symptoms	Causes and Comments
Lower than expected concentration of nucleic acid in eluate (a typical FFPE section should yield amplifiable nucleic acid depending on tissue size, cellularity, formalin fixation condition and handling)	Kit performance has been evaluated by isolating nucleic acid from FFPE tissue samples up to 80µm in total thickness with a maximum individual section thickness of 20µm. Only use sections that meet the size specification.
	The kit was designed for use with FFPE tissue samples. It was not designed for use with nonFFPE tissue samples, such as fresh or frozen tissue samples. Incubation times and temperatures were tested to ensure optimal yield.
	The kit was not designed for use with tissue samples that have been prepared with fixatives other than 10% neutral-buffered formalin. Check with the pathology lab or vendor to ensure that an alternative fixative was not used.
	No claims are made for stained slides or sections. Repeat the extraction with an unstained slide or section.
	Kit performance was evaluated based upon extracting amplifiable nucleic acid and fluorescent dye binding. Other quantitation methods including absorbance may not correlate with amplification and fluorescence.
	RNases or DNases may have been introduced during sample processing or quantitation. See Section 9 for information on creating a ribonuclease-free environment.
	DNase cocktail was added to the cartridge for the incorrect workflow. DNase cocktail should only be added to well #7 of the cartridge for the RNA and Sequential RNA workflows.
	Isopropanol was not added to the cartridge for the appropriate workflow (RNA, TNA or Sequential RNA), or was added to the wrong well of the cartridge.

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8. Troubleshooting (continued)

Symptoms	Causes and Comments
Lower than expected concentration of nucleic acid in eluate (a typical FFPE section should yield amplifiable nucleic acid depending on tissue size, cellularity, formalin fixation condition and handling) (continued)	The incorrect Maxwell® method was used for the instrument extraction run. Confirm that the Maxwell® method used matches the cartridge preparation for the workflow used.
	Nuclease-Free Water was not added to the elution tubes or the incorrect volume was added to the elution tubes. The kit was tested with a 30–100µl elution volume
Lower than expected quality (the eluate contains highly fragmented nucleic acid or inhibitors of downstream assays)	The tissue section used for extraction may include fragmented nucleic acid due to formalin fixation conditions or handling. If the nucleic acid is fragmented prior to the extraction method, fragmented nucleic acid will be purified with this kit. Repeat with an adjacent section to assess whether there is a problem with the section or with the extraction process.
	Some amplification assays are particularly sensitive to inhibitors. Downstream assay controls should identify the presence of an amplification inhibitor in the eluate. You are responsible for verifying the compatibility of this product with all downstream assays.
	The presence of multiple nucleic acid types (DNA and RNA) in an eluate can cause competition in downstream assays. In the case of competition, optimize the assay for the analyte of interest.
DNA present in RNA eluates (RNA eluates are contaminated with DNA, which may interfere with downstream assays.)	The DNase cocktail was not added to the cartridge for the appropriate workflow (RNA or Sequential RNA), or was added to the wrong well of the cartridge.
	The elution tube was not removed from the deck tray and replaced with a new elution tube and elution buffer when running the Sequential DNA/RNA workflow.
RNA present in DNA eluates (DNA eluates are contaminated with RNA, which may interfere with downstream assays.)	RNase can be added to the DNA eluates to remove any RNA present in DNA samples if RNA-free DNA is required.
DNA method is accidentally or intentionally aborted during the sequential workflow.	RNA samples can be recovered by running the cartridge with the Maxwell® RSC XtractAll FFPE DNA/RNA Kit (RNA) method.

Symptoms	Causes and Comments
Resin carryover into eluates	Undigested material was transferred into the cartridge. If any undigested material remains at the end of incubation, centrifuge sample tubes at 10,000 × *g* for 20 seconds to pellet any undigested material. Do not transfer any pelleted or undigested material to the cartridge.
	Some resin carryover is normal and does not affect downstream performance. If necessary, use an Elution Magnet ([Cat.# AS4017, Cat.# AS4018 or both]; available separately) to transfer the eluate into a new tube. See Section 10, Related Products.
Samples were accidentally exposed to UV treatment inside the instrument, e.g., between sequential extraction of DNA and RNA	If the Maxwell® Instrument Sanitization setting for "Sanitize after extraction" is turned ON, then the UV treatment will be automatically performed after the XtractAll FFPE (Sequential) extraction method has completed and the "Close Door" option has been selected. To minimize risk of accidental exposure of samples to UV irradiation, before starting the protocol for sequentially extracting DNA and RNA, ensure that the Sanitization Setting for "Sanitize after extraction" on your Maxwell® Instrument is turned OFF. Please refer to the Technical Manual for your Maxwell® Instrument for details on managing the Sanitization Settings (see Table 1).

9. Creating a Ribonuclease-Free Environment

Ribonucleases are extremely difficult to inactivate. Take care to avoid introducing RNase activity into your RNA samples during and after isolation. This is especially important if the starting material is only available in a limited quantity. The following notes may help prevent accidental RNase contamination of your samples.

1. Two of the most common sources of RNase contamination are the user's hands and bacteria or molds that may be present on airborne dust particles. To prevent contamination from these sources, use aseptic technique when handling the reagents supplied with this system. Wear gloves at all times. Change gloves whenever ribonucleases may have been contacted.
2. Whenever possible, use sterile, disposable plasticware for handling RNA. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
3. Treat nonsterile glassware and plasticware before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plasticware with 0.1N NaOH, 1mM EDTA, followed by RNase-free water. Commercially available RNase removal products also may be used, following the manufacturer's instructions.

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9. Creating a Ribonuclease-Free Environment (continued)

4. Treat solutions not supplied with the system by adding diethyl pyrocarbonate (DEPC) to 0.1% in a fume hood. Incubate overnight with stirring at room temperature in the hood. Autoclave for 30 minutes to remove any trace of DEPC.

   Caution: DEPC is a suspected carcinogen and should only be used in a chemical fume hood. DEPC reacts rapidly with amines and cannot be used to treat Tris buffers.

   Note: For all downstream applications, it is essential that you continue to protect your RNA samples from RNases.

10. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument*	1 each	AS4500
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC 48 Instrument*	1 each	AS8500
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
RSC/CSC Plungers	50/pack	AS1331
Maxwell® RSC Plunger Pack	48/pack	AS1670
Maxwell® FSC Instrument**	1 each	AS4600
Maxwell® FSC Deck Tray	1 each	AS4016
Microtube, 1.5ml	1,000/pack	V1231
Elution Magnet, 16 Position	1 each	AS4017
Elution Magnet, 24 Position	1 each	AS4018

*For Research Use Only. Not for Use in Diagnostic Procedures.

**Not For Medical Diagnostic Use.

Maxwell® RSC Reagent Kits

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

11. Summary of Changes

The following changes were made to the 7/25 revision of this document:

1. Updated the recommended software version for the Maxwell® RSC 48 or CSC 48 (RUO mode) from version 4.1.0 to new software version 4.1.1 on the cover page and in Section 5.
2. Corrected a subsection heading on p. 12 from 6.E to 4.E.
3. Added warning text to Sections 4.E and 8, noting that the "Sanitation after extraction" setting must be set to OFF before running XtractAll FFPE DNA (Sequential) extraction method, to avoid exposing samples to UV light. See the Technical Manual for your Maxwell® Instrument for details on how to manage this setting.