TECHNICAL MANUAL

Maxwell® RSC RNA FFPE Kit

Instructions for Use of Product
AS1440

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

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

Revised 11/17
TM436

Maxwell® RSC RNA FFPE 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

Promega

1. Description

The Maxwell® RSC RNA FFPE Kit[a] is used with the Maxwell® RSC or Maxwell® RSC 48 Instrument to provide a simple method for efficient, automated purification of RNA from FFPE (formalin-fixed, paraffin-embedded) mammalian tissue samples. The Maxwell® RSC and Maxwell® RSC 48 Instruments are supplied with preprogrammed purification procedures and are designed for use with predispensed reagent cartridges, maximizing simplicity and convenience. The Maxwell® RSC Instrument can process up to 16 samples, and the Maxwell® RSC 48 Instrument can process up to 48 samples in about 40 minutes. The purified RNA can be used directly in downstream amplification-based assays such as RT-PCR.

The Maxwell® RSC RNA FFPE Kit purifies samples using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of RNA. The Maxwell® RSC and Maxwell® RSC 48 Instruments are magnetic particle-handling instruments that efficiently bind RNA to the paramagnetic particle in the first well of a prefilled cartridge. The samples are processed through a series of washes before the RNA is eluted.

Prior to extraction, samples can be preprocessed manually or using the Maxprep™ Liquid Handler. The Maxprep™ Liquid Handler will prepare samples for preprocessing in tubes and can add preprocessed samples from sample tubes to Maxwell® FFPE Cartridges, transfer plungers to Maxwell® FFPE Cartridges and dispense elution buffer to elution tubes. Follow the instruction set specific to the preprocessing option used.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC RNA FFPE Kit	**48 preps**	**AS1440**

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

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

Storage Conditions: Store the Maxwell® RSC RNA FFPE Kit at ambient temperature (15–30°C). Store reconstituted DNase I at –30°C to –10°C.

Safety Information: The Maxwell® FFPE Cartridges contain ethanol, isopropanol and guanidine hydrochloride. Ethanol and isopropanol should be considered flammable, harmful and irritants. Guanidine hydrochloride should be considered toxic, harmful and an irritant. Refer to the SDS for detailed safety information.

Maxwell® FFPE Cartridges are designed to be used with 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.

For Preprocessing with the Maxprep™ Liquid Handler

PRODUCT	SIZE	CAT.#
Nunc 2.0ml Deep Well Plates	60/pack	AS9307
Maxprep™ 1000µl Conductive Disposable Tips, Filtered	40/box	AS9303
Maxprep™ 300µl Conductive Disposable Tips, Filtered	60/box	AS9302
Maxprep™ Reagent Reservoir, 50ml	28/pack	AS9304
Maxwell® RSC Plunger Pack	1 each	AS1670
Maxprep™ Plunger Holder	1 each	AS9408
Maxprep™ 3-Position Reagent Tube Holder	1 each	AS9409

3. Sample Preparation

Materials to Be Supplied By the User

- microcentrifuge
- 1.5–2.0ml tubes for incubation of samples (e.g., Microtubes, 1.5ml; Cat.# V1231)
- FFPE tissue sections (5–10 microns thick with a size range of 20mm^2 to 200mm^2 for a total of up to 2.0mm^3 of tissue) **Note:** Store samples at room temperature (15–30°C).
- razor blades (**Note:** Use caution when using razor blades to scrape samples from slides.)

3.A. Sample Information

The Maxwell® RSC RNA FFPE Kit is only intended for use with FFPE tissue samples. It is not intended for use with non-FFPE tissue samples, such as fresh or frozen tissue samples.

The Maxwell® RSC RNA FFPE Kit is not intended for use with tissue samples prepared with fixatives other than 10% neutral-buffered formalin.

The Maxwell® RSC RNA FFPE Kit performance has been evaluated by isolating RNA from FFPE mammalian (mouse and human) tissue samples ranging in thickness from 5–10 microns with a size range of 20mm^2 to 200mm^2 for a total of up to 2.0mm^3.

3.B. Preparation of DNase I

Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I prior to use. Invert the vial to rinse DNase I off the underside of the cap and swirl gently to mix; do not vortex. Store reconstituted DNase I at –30°C to –10°C after use. DNase I solution maintains activity for up to 10 freeze-thaw cycles.

3.C. Preparation of FFPE Samples

Place the FFPE tissue section into a 1.5ml or 2.0ml microcentrifuge tube. If using slide-mounted tissue sections, scrape section off the slide using a clean razor blade. Centrifuge the tube at maximum speed for 15 seconds to collect the sample at the bottom of the tube.

Note: Tissue sections ranging in thickness from 5–10 microns thick with a size range of 20mm^2 to 200mm^2 for a total of up to 2.0mm^3 can be used, if necessary.

4. Manual Preprocessing

4.A. Preprocessing of FFPE Section Samples

Materials to Be Supplied by the User

- microcentrifuge
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- heating blocks set to 56°C and 80°C

1. Add 300µl of Mineral Oil to the sample tubes. Vortex for 10 seconds.
2. Heat the samples at 80°C for 2 minutes. Place the samples at room temperature while the master mix is prepared.
3. Prepare a master mix of the Lysis Buffer, Proteinase K Solution and Blue Dye as shown below:

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

For fewer than six samples, prepare enough master mix for n + 1 samples.
Note: Use the master mix within 1 hour of preparation. Master mix cannot be stored for later use.

4. Add 250µl of master mix to each sample tube, and vortex for 5 seconds.
5. Centrifuge at 10,000 × *g* for 20 seconds to separate layers. If a pellet is present in the aqueous layer (lower, blue layer), gently mix aqueous phase with a pipette.
6. Transfer the sample tubes to a 56°C heat block and incubate for 15 minutes.
7. Transfer the sample tubes to an 80°C heat block and incubate for 1 hour.
8. Remove the sample tubes from the heat block, and allow the samples to cool to room temperature for 15 minutes.

9. Prepare a DNase cocktail containing $MnCl_2$, DNase Buffer and reconstituted DNase I (see Section 3.B for DNase I preparation) in the order shown below:

Reagent	Amount/Reaction	Reactions (number to be run + 2)	Total
$MnCl_2$, 0.09M	26µl	n + 2	26 × (n + 2)µl
DNase Buffer	14µl	n + 2	14 × (n + 2)µl
DNase I	10µl	n + 2	10 × (n + 2)µl

For fewer than six samples, prepare enough master mix for n + 1 samples.

Notes:

1. If DNase cocktail reagents are added individually to sample tubes, be certain to add them in the order shown. Incorporate each reagent by thorough pipetting.
2. Store remaining reconstituted DNase I at −30°C to −10°C.
3. DNase Buffer can precipitate if stored below freezing for extended periods of time. If the buffer has precipitated, it can be solubilized by heating to 56°C for 2 minutes, followed by vortexing briefly to mix.

10. Add 50µl of DNase cocktail to the aqueous (blue) phase in each sample tube. Mix by pipetting 10 times.
11. Incubate sample tubes for 15 minutes at room temperature (15–30°C). During the incubation refer to Section 4.B to begin cartridge preparation.
12. Centrifuge the sample tubes at full speed in a microcentrifuge for 2 minutes.
13. Immediately transfer the blue, aqueous phase to well #1 of a Maxwell® FFPE Cartridge.

    Note: For some difficult sample types (e.g., skin) incubating the sample in well #1 for 5 minutes before starting the Maxwell® method may increase RNA recovery.

4.B. Maxwell® FFPE Cartridge Preparation

1. To maintain an RNase-free environment during processing, change gloves before handling Maxwell® FFPE Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used 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. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s).
4. Add 50µl of Nuclease-Free Water to the bottom of each elution tube.

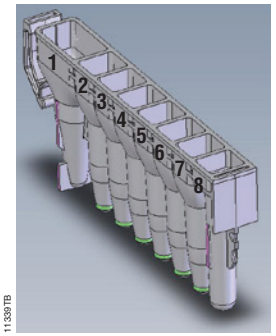
   Note: Only use the Nuclease-Free Water provided in the Maxwell® RSC RNA FFPE Kit. Use of other elution buffers may impact RNA purification.

4.B. Maxwell® FFPE Cartridge Preparation (continued)

5. Proceed to Section 6, Maxwell® Instrument Setup and Run.

Notes:

1. 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 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® Instrument.



User Adds to Wells

1. Sample lysates

8. RSC Plunger

Figure 1. Maxwell® FFPE Cartridge contents.

Figure 2. Setup and configuration of the deck trays. Nuclease-Free Water is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

5. Maxprep™ Preprocessing

Materials to Be Supplied by the User

- heating blocks set to 56°C and 80°C

5.A. FFPE Lysis Method Run (First Method)

Note: FFPE samples are processed through two preprocessing methods on the Maxprep™ Liquid Handler. The first preprocessing method prepares and dispenses the lysis mix to the FFPE samples in 1.5ml or 2.0ml tubes. After this the user will remove the tubes from the Maxprep™ Liquid Handler for incubation at 56°C for 15 minutes and incubation at 80°C for 1 hour.

1. Turn on the Maxprep™ Liquid Handler and PC. Log in to the PC, and start the Maxprep™ software on the PC by double-clicking the desktop icon.
2. Touch **Start** to access the 'Methods' screen.
3. On the 'Methods' screen, touch the FFPE Lysis Reaction Preparation preprocessing method or laboratory-specific variant of the FFPE Lysis Reaction Preparation preprocessing method.
4. Verify that the appropriate preprocessing method or variant method has been selected, and touch the **Proceed** button. Close the instrument door and touch the **Run** button on the method run screen to start the run.
5. When prompted, enter the sample number.
6. Follow instrument setup instructions displayed in the method. You will be directed by the Maxprep™ software where to place the following items on the instrument:
    - Maxprep™ 3-Position Reagent Tube Holder with up to 3 Proteinase K Solution tubes
    - Maxprep™ Reagent Reservoir, 50ml with Lysis Buffer
    - Maxprep™ Reagent Reservoir, 50ml with Mineral Oil
    - 10mm diameter tube carriers with FFPE sections in 1.5ml flip-cap or 2.0ml screw-cap tubes (All tubes within a carrier must be of the same type)
    - Nunc 2.0ml Deep Well Plate
    - Maxprep™ 1000µl Conductive Disposable Tips, Filtered (2; one rack may be partially full)
    - Maxprep™ 300µl Conductive Disposable Tips, Filtered (rack may be partial or full)
7. Close the instrument door, and touch the **Next** button to start the automated preprocessing setup of samples.

5.B. Maxprep™ Liquid Handler Preprocessing Protocol (FFPE Lysis)

The Maxprep™ Liquid Handler will prepare samples prior to lysis incubations. The following steps are performed by the Maxprep™ Liquid Handler:

1. Mineral Oil is transferred to the Nunc 2.0ml Deep Well Plate for heating.
2. The system prepares a lysis reaction in the input sample tubes consisting of the following components:
   - 25µl of Proteinase K Solution
   - 224µl of Lysis Buffer
   - 300µl of heated Mineral Oil

   Note: While not necessary for automated processing, you can optionally add Blue Dye solution to the Lysis Buffer that is placed on the system at a ratio of 1µl of Blue Dye to each 224µl of Lysis Buffer.
3. Method is complete. Open instrument door and remove the sample tubes. Remove used tips from the waste bin and discard as hazardous waste following your institution's recommended guidelines. Either discard or tightly cap and store remaining reagents.

Consumables for Maxprep™ preprocessing methods are designed to be used with potentially infectious substances. Use appropriate protective equipment (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.

5.C. FFPE Sample Incubation

After addition of lysis components to the sample tubes containing FFPE sections, remove sample tubes from the Maxprep™ Liquid Handler and perform the following incubation steps for all tubes:

1. Centrifuge the sample tubes at maximum speed in a microcentrifuge for 90 seconds.
2. Transfer the sample tubes to a 56°C heat block and incubate for 15 minutes.
3. Transfer the sample tubes to an 80°C heat block and incubate for 1 hour.
4. Place the sample tubes back into the 10mm diameter tube carriers for the second Maxprep™ preprocessing method.

5.D. Maxprep™ Cartridge Preparation (Second Method)

Samples are returned to the system for the second preprocessing method that will perform DNase treatment, deck tray preparation and sample transfer to cartridges.

Prior to starting the second method:

Prepare a DNase Cocktail containing $MnCl_2$, DNase Buffer and reconstituted DNase I (see Section 3.B for DNase I preparation) in the order shown in the following table:

Reagent	Amount/Reaction	Reactions (number to be run + 2)	Total
$MnCl_2$, 0.09M	26µl	n + 2	26 × (n + 2)µl
DNase Buffer	14µl	n + 2	14 × (n + 2)µl
DNase I	10µl	n + 2	10 × (n + 2)µl

Notes:

1. Create the DNase cocktail in a tube large enough to accommodate the entire volume. After preparation, aliquot the DNase cocktail to one to three 1.5ml flip-cap tubes for placement on the system.
2. Store remaining reconstituted DNase I at –30°C to –10°C.
3. DNase Buffer can precipitate if stored below freezing for extended periods of time. If the buffer has precipitated, it can be solubilized by heating to 56°C for 2 minutes, followed by vortexing briefly to mix.

1. Turn on the Maxprep™ Liquid Handler and PC. Log in to the PC, and start the Maxprep™ software on the PC by double-clicking the desktop icon.
2. Touch **Start** to access the 'Methods' screen.

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

   a. Touch the Maxwell® RSC RNA FFPE preprocessing method or laboratory-specific variant of the Maxwell® RSC RNA FFPE preprocessing method.

   b. Use a bar code reader to scan the 2D bar code on the kit box to filter the available methods for the Maxwell® RSC RNA FFPE Kit. Touch the Maxwell® RSC RNA FFPE preprocessing method or laboratory-specific variant of the Maxwell® RSC RNA FFPE preprocessing method if desired.
3. Verify that the appropriate preprocessing method or variant method has been selected and touch the **Proceed** button. Close the instrument door and touch the **Run** button to start the run.
4. Enter any method-specific variables (Sample Number, Elution Volume).
5. Prior to placing Maxwell® RSC or Maxwell® RSC 48 Deck Tray(s) on the instrument, prepare the deck tray(s) with cartridges and elution tubes. Change gloves before handling Maxwell® FFPE Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used 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. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s).

   Notes:

   1. 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 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® Instrument.

5.D. Maxprep™ Cartridge Preparation (Second Method) (continued)

6. Follow instrument setup instructions displayed in the method. You will be directed by the Maxprep™ software where to place the following items on the instrument:
   - Maxprep™ Plunger Holders with Maxwell® RSC Plunger Packs (2; one may be partially filled)
   - Maxwell® RSC 48 Front Deck Tray or Maxwell® RSC Deck Tray containing Maxwell® FFPE Cartridges with seals removed and open elution tubes
   - Maxprep™ 3-Position Reagent Tube Holder with up to 3 DNase Cocktail Tubes
   - Maxprep™ Reagent Reservoir, 50ml with Nuclease-Free Water
   - 10mm diameter tube carriers with 1.5ml flip-cap or 2.0ml screw-cap tubes containing centrifuged FFPE sections (all tubes within a carrier must be of the same type)
   - Maxprep™ 1000µl Conductive Disposable Tips, Filtered (2; one rack may be partially full)
   - Maxprep™ 300µl Conductive Disposable Tips, Filtered (rack may be partial or full)
7. Close the instrument door, and touch the **Next** button to start the automated preprocessing of samples.

5.E. Maxprep™ Liquid Handler Preprocessing Protocol (RNA FFPE)

The Maxprep™ Liquid Handler will prepare samples prior to extraction using the Maxwell® RSC or Maxwell® RSC 48 Instrument. The following steps are performed by the Maxprep™ Liquid Handler:

1. The system transfers 50µl of DNase I cocktail to the aqueous phase of the sample lysate and incubates at room temperature (15–30°C) for 15 minutes.
2. Plungers are transferred to each of the cartridges in the Maxwell® RSC or Maxwell® RSC 48 Deck Tray(s). The specified volume of Nuclease-Free Water is transferred to the elution tubes for each position in the Maxwell® RSC or Maxwell® RSC 48 Deck Tray(s).
3. The system transfers the sample lysate from each sample tube to its corresponding Maxwell® FFPE cartridge.
4. Method is complete. Open instrument door and move the deck tray(s) to the Maxwell® RSC or Maxwell® RSC 48 Instrument for extraction. Remove primary sample tubes and used tips from the waste bin of the instrument, and discard as hazardous waste following your institution's recommended guidelines. Either discard or tightly cap and store remaining reagents.

Consumables for Maxprep™ preprocessing methods are designed to be used with potentially infectious substances. Use appropriate protective equipment (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.

6. Maxwell® Instrument Setup and Run

Refer to the *Maxwell® RSC Instrument Operating Manual* #TM411 or *Maxwell® RSC 48 Instrument Operating Manual* #TM510 for detailed information.

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 power up, proceed through a self test and home all moving parts.
2. Touch **Start** to access the 'Methods' screen. When running in Portal mode, scan the bar code on the deck tray(s). After data has been returned from the Portal database, 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.
3. On the 'Methods' screen, if a method has not been selected by scanning the bar code on the deck trays, select a method using one of the two options below:
   a. Touch the RNA FFPE method.
   b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate method.
4. Verify that the RNA FFPE method has been selected, and touch the **Proceed** button. If requested by the software, enter any kit lot and expiration information required by the Administrator.
5. On the 'Cartridge Setup' screen (if shown), touch the cartridge positions to select/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, use 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: 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: When using the Maxwell® RSC 48 Instrument, check the identifier on the Maxwell® RSC 48 Deck Tray to determine whether it should be placed in the front or back of the instrument.

6. 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.

   Note: When using the Maxwell® RSC 48 Instrument, if the Vision System has been enabled, the deck tray(s) 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.

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. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.
2. 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.

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 *Maxwell® RSC Instrument Operating Manual* or the *Maxwell® RSC 48 Instrument Operating Manual* 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 RNA, and cap the tubes. If paramagnetic particles are present in the elution tubes, centrifuge at 10,000–20,000 × *g* for 2–5 minutes. After the run is complete, the extraction run report will be displayed. From the 'Report View' screen, you can print or export this report or both.
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.

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

7. Recommendations for Quantitation

Determine whether the purified RNA sample yield and purity meets the input requirements for the appropriate downstream assay prior to use in that assay. Kit performance was evaluated based upon the purification of amplifiable RNA. Other means of quantitation including absorbance or fluorescent dye binding, may not correlate with amplification (1). Absorbance readings for purified FFPE samples may overestimate yield; we recommend using other methods for determining yield (1).

8. 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 concentration of RNA in eluate	Kit performance has been evaluated by isolating RNA from 5–10µM thick FFPE tissue samples ranging in size from 0.1mm^3 to 2.0mm^3. It was not designed for samples outside this range.
	The kit is intended for use with FFPE mammalian tissue samples. It is not intended for use with non-FFPE tissue samples, such as fresh or frozen tissue samples or with FFPE tissue samples collected from non-mammalian tissues.
	The kit is not intended for use with tissue samples prepared with fixatives other than 10% neutral-buffered formalin.
	No claims are made for stained slides or sections. Repeat the purification with an unstained slide or section.
	Kit performance was evaluated based upon the purification of amplifiable RNA. Other means of quantitation including absorbance or fluorescent dye binding may not correlate with amplification. Use an amplification quantitation method to assess yield.
Lower than expected quality (the eluate contains highly fragmented RNA or inhibitors of downstream assays)	Formalin fixation and subsequent crosslink reversal will fragment RNA. If the RNA is fragmented prior to extraction and purification, fragmented RNA will be purified with this kit. Repeat with an adjacent section to assess whether the fragmentation is inherent to the sample or if the RNA is fragmented during purification.
	Some amplification assays are particularly sensitive to the presence of inhibitors. Downstream assay controls should identify the presence of an amplification inhibitor in the eluate. It is the user's responsibility to verify the compatibility of this product with downstream assays.

8. Troubleshooting (continued)

Symptoms	Causes and Comments
DNA present in eluates	The DNase cocktail added to the sample provides an excess of DNase activity when used with FFPE tissue samples ranging in size from 0.1mm^3 to 2.0mm^3. The kit is not designed for samples outside this range. Use sample amounts that will fall within this range.
	The sample must be cooled to room temperature before the DNase cocktail is added. Do not cool the sample on ice. High or low temperature will result in poor DNase activity.
	Insufficient mixing of the DNase cocktail into the sample during preprocessing can result in incomplete degradation of DNA. Be sure to mix the DNase cocktail thoroughly into the sample.
	If the DNase cocktail components are added to the sample separately, be sure to add them in the order indicated in Section 4.A, Step 9. In addition, be sure to mix each component thoroughly as it is added. Adding the components in a different order or mixing incompletely can inactivate DNase.

9. Creating a Ribonuclease-Free Environment

Ribonucleases (RNases) 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 was difficult to obtain or is irreplaceable. 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 sterile 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 plastic ware for handling RNA. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
3. Treat nonsterile glassware, plasticware and electrophoresis chambers 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.

   Note: Electrophoresis chambers may be contaminated with ribonucleases, particularly RNase A, from analysis of DNA samples. Whenever possible, set aside a new or decontaminated apparatus for RNA analysis only.

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. Continue to wear clean gloves and use solutions and centrifuge tubes that are RNase-free.

10. Reference

1. Bonin, S. *et al*. (2010) Multicentre validation study of nucleic acids extraction from FFPE tissues. *Virchows Arc*. **425**, 309–17.

11. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC Plunger Pack	1 each	AS1670
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
Maxprep™ Carrier, Maxwell® RSC	1 each	AS9402
Maxprep™ Carrier, Maxwell® RSC 48 Front	1 each	AS9403
Maxprep™ Carrier, Maxwell® RSC 48 Back	1 each	AS9404
Maxprep™ Liquid Handler, RSC Carriers	1 each	AS9100
Maxprep™ Liquid Handler, RSC Carriers w/UV light	1 each	AS9101
Maxprep™ Liquid Handler, RSC 48 Carriers	1 each	AS9200
Maxprep™ Liquid Handler, RSC 48 Carriers w/UV light	1 each	AS9201
Maxprep™ 1000µl Conductive Disposable Tips, Filtered	40/box	AS9303
Maxprep™ 300µl Conductive Disposable Tips, Filtered	60/box	AS9302
Maxprep™ Reagent Reservoir, 50ml	28/pack	AS9304
Maxprep™ Waste Bags, Clear	100/box	AS9305
Nunc 2.0ml Deep Well Plates	60/pack	AS9307
Maxprep™ Plunger Holder	1 each	AS9408
Maxprep™ 3-Position Reagent Tube Holder	1 each	AS9409

Maxwell® RSC Reagent Kits

For a list of available Maxwell® RSC purification kits, visit: **www.promega.com**

12. Summary of Changes

The following changes were made to the 11/17 revision of this document:

1. Instructions were added for preprocessing with the Maxprep™ Liquid Handler.
2. Instructions were added for processing with the Maxwell® RSC 48 Instrument.

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