

TECHNICAL MANUAL

# Maxwell® RSC miRNA Tissue Kit

Instructions for Use of Product **AS1460** 

**Note:** To use the Maxwell<sup>®</sup> RSC miRNA Tissue Kit, you must have the "miRNA Tissue" method loaded on the Maxwell<sup>®</sup> Instrument.

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



# Maxwell® RSC miRNA Tissue 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

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# 1. Description

The Maxwell<sup>®</sup> RSC miRNA Tissue Kit<sup>(a)</sup> is designed for purification of total RNA, including microRNA (miRNA), from tissue samples. The miRNA Tissue procedure purifies total RNA with minimal sample handling before automated purification on the Maxwell<sup>®</sup> Instruments specified in Table 1. The Maxwell<sup>®</sup> Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification methods, maximizing simplicity and convenience. The Maxwell<sup>®</sup> methods for the RSC miRNA Tissue Kit can process from one to the maximum sample number in about 70 minutes. The low elution volume results in concentrated high-quality RNA suitable for use in downstream applications such as quantitative RT-PCR (qRT-PCR).

# Table 1. Supported Instruments.

Instrument	Cat.#	<b>Operating Manual</b>	
Maxwell <sup>®</sup> RSC	AS4500	TM411	
Maxwell <sup>®</sup> RSC 48	AS8500	TM510	
Maxwell® CSC RUO Mode	AS6000	TM573	
Maxwell <sup>®</sup> CSC 48 RUO Mode	AS8000	TM628	
Maxprep <sup>™</sup> Liquid Handler	AS9100, AS9101, AS9200, AS9201	TM509	

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

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

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#### 2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell <sup>®</sup> RSC miRNA Tissue Kit	48 preps	AS1460

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

- 30ml Homogenization Solution
- 20ml Lysis Buffer
- 20ml Lytic Enhancer
- 2 vials Proteinase K (PK) Solution
- 900µl 1-Thioglycerol
- 2 vials DNase I (lyophilized)
- 50µl Blue Dye
- 48 Maxwell<sup>®</sup> RSC Cartridges (RSCN)
- 1 Maxwell<sup>®</sup> RSC Plunger Pack (48 plungers)
- 50 Elution Tubes (0.5ml)
- 25ml Nuclease-Free Water

**Storage Conditions:** Upon receipt, remove the 1-Thioglycerol and store at  $+2^{\circ}$ C to  $+10^{\circ}$ C. Store the remaining kit components at room temperature ( $+15^{\circ}$ C to  $+30^{\circ}$ C). 1-Thioglycerol also can be stored at room temperature ( $+15^{\circ}$ C to  $+30^{\circ}$ C), where it is stable for up to 9 months. Store rehydrated DNase I at  $-30^{\circ}$ C to  $-10^{\circ}$ C. Do not subject DNase I Solution to more than 10 freeze-thaw cycles.

**Safety Information:** The Maxwell<sup>®</sup> RSC Cartridges contain ethanol and isopropanol, which are flammable and irritants. 1-Thioglycerol is toxic. Guanidine thiocyanate and guanidine hydrochloride (which are components of the Homogenization Solution and Lysis Buffer) are toxic, harmful and irritants. Wear gloves and follow standard safety procedures while working with these substances. Refer to the SDS for detailed safety information.



Maxwell<sup>®</sup> RSC 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.



**Note:** Bleach reacts with guanidine thiocyanate and should not be added to any sample waste containing the Homogenization Solution.

## For Preprocessing with the Maxprep<sup>™</sup> Liquid Handler

PRODUCT	SIZE	CAT.#
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 <sup>®</sup> RSC Plunger Pack	48/pack	AS1670
Maxprep™ Plunger Holder	1 each	AS9408
Maxprep™ 3-Position Reagent Tube Holder	1 each	AS9409



# 3. Sample Preparation

D The Maxwell<sup>®</sup> RSC miRNA Tissue Kit will produce optimal results with 10mg of most tissues. Up to 20mg of some tissues (e.g., heart) may result in higher yields.

# 3.A. Preparation of Solutions

# 1-Thioglycerol/Homogenization Solution

A volume of 200 $\mu$ l of 1-Thioglycerol/Homogenization Solution is needed for each sample. To prepare a working solution, add 20 $\mu$ l of 1-Thioglycerol per milliliter of Homogenization Solution. 1-Thioglycerol is viscous, so careful pipetting is required for accurate measurement. We recommend adding 600 $\mu$ l of 1-Thioglycerol to the 30ml bottle of Homogenization Solution. Before use, chill the 1-Thioglycerol/Homogenization Solution on ice or at 2–10°C.

Note: Store the 1-Thioglycerol/Homogenization Solution at 2-10°C, where it is stable for up to 30 days.

## **DNase I Solution**

Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I. Invert to rinse DNase off the underside of the cap and swirl gently to mix; do not vortex. Add 5µl of Blue Dye to the reconstituted DNase I as a visual aid for pipetting. Dispense the DNase I Solution into single-use aliquots in nuclease-free tubes. Each purification requires 10µl of DNase I Solution. Store reconstituted DNase I Solution at  $-30^{\circ}$ C to  $-10^{\circ}$ C. DNase I Solution maintains activity for up to 10 freeze-thaw cycles. When using DNase I Solution on a Maxprep<sup>™</sup> Liquid Handler, store in a 1.5ml microcentrifuge tube.

## 3.B. Creation of Tissue Lysates

## Materials to Be Supplied By the User

- small tissue homogenizer (e.g., Tissue-Tearor<sup>™</sup> homogenizer [PRO Scientific], or any homogenizer capable of handling small volumes)
- benchtop vortex mixer
- 1.5ml-2.0ml tube for homogenization
- RNase-free, sterile, aerosol resistant pipette tips

Working as quickly as possible, homogenize the tissue sample in the chilled 1-Thioglycerol/Homogenization Solution until no visible tissue fragments remain. Homogenize for an additional 15–30 seconds to ensure complete homogenization. If foaming occurs, let the sample settle on ice. Extra 1-Thioglycerol/Homogenization Solution is provided, but only 200µl of homogenate can be processed per cartridge. The final volume of the homogenate to be added to the cartridge should be 200µl. Add more 1-Thioglycerol/Homogenization Solution as needed to bring the sample to a final volume of 200µl.

**Note:** After homogenization, samples may be stored frozen at -80°C for later processing. Thaw frozen homogenates on ice or at 2–10°C to avoid RNA degradation.

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# 4. Manual Preprocessing

# 4.A. Preprocessing of Tissue Samples

- 1. Add 200µl of Lysis Buffer (Part# MC501C), 200µl of Lytic Enhancer (Part# MC145A) and 30µl of Proteinase K to the homogenized sample. Mix by vortexing for 20 seconds.
- 2 Incubate at room temperature for 10 minutes. During this time, prepare the Maxwell<sup>®</sup> RSC Cartridges as described in Section 4.B.
- 3. Transfer 630µl of lysate to well #1 (the largest well in the cartridge) of the Maxwell® RSC Cartridge (RSCN).
- 4. Add 10µl of blue DNase I Solution (prepared in Section 3.A) to well #4 of the Maxwell® RSC miRNA Tissue Cartridge (well #4 contains yellow reagent). After the blue DNase I Solution is added, the reagent in well #4 will be green.
- 5. Proceed to Section 6 for instructions on loading samples onto the instrument and beginning the automated purification run.

# 4.B. Maxwell<sup>®</sup> RSC miRNA Tissue Cartridge (RSCN) Preparation

Cartridges should be prepared shortly before adding the lysate at Step 4 in Section 4.A.

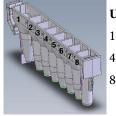
- 1. To maintain an RNase-free environment during processing, change gloves before handling Maxwell® RSC Cartridges (RSCN), RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) facing away from the elution tubes. 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. Add 60µl of Nuclease-Free Water to the bottom of each elution tube.

#### 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<sup>®</sup> Instrument.



User Adds to Wells

1. Sample lysates 4. DNase I Solution

8. RSC Plunger

# Figure 1. Maxwell<sup>®</sup> RSC Cartridge (RSCN) 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<sup>™</sup> Preprocessing

## 5.A. Maxprep<sup>™</sup> Cartridge Preparation

- 1. Turn on the Maxprep<sup>™</sup> Liquid Handler and PC. Log in to the PC, and start the Maxprep<sup>™</sup> software on the PC by double-clicking the desktop icon.
- 2. Touch **Start** to access the 'Methods' screen.
- 3. On the 'Methods' screen, select a method using one of the two options below:
  - a. Touch the miRNA Tissue preprocessing method or laboratory-specific variant of the miRNA Tissue preprocessing method.
  - b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate base method. Touch the laboratory-specific variant of the miRNA Tissue preprocessing method if desired.
- 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. Enter any method-specific variables (Sample Number, Elution Volume).
- 6. Prior to placing Maxwell<sup>®</sup> deck tray(s) on the instrument, prepare the deck tray(s) with cartridges and elution tubes. Change gloves before handling Maxwell<sup>®</sup> RSC Cartridges (RSCN), 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<sup>®</sup> Instrument.



# 5.A. Maxprep<sup>™</sup> Cartridge Preparation (continued)

- 7. Follow instrument setup instructions displayed in the method. You will be directed by the Maxprep<sup>™</sup> software where to place the following items on the instrument:
  - Maxprep<sup>™</sup> Plunger Holders with Maxwell<sup>®</sup> RSC Plunger Packs (2; one may be partially full)
  - 24-position Maxwell<sup>®</sup> Front Deck Tray or 16-position Maxwell<sup>®</sup> Deck Tray containing Maxwell<sup>®</sup> RSC Cartridges (RSCN) with seals removed and open elution tubes
  - 24-position Maxwell<sup>®</sup> Back Deck Tray or 16-position Maxwell<sup>®</sup> Deck Tray containing Maxwell<sup>®</sup> RSC Cartridges (RSCN) with seals removed and open elution tubes
  - Maxprep<sup>™</sup> 3-Position Reagent Tube Holder with up to 3 Proteinase K Tubes
  - Maxprep<sup>™</sup> 3-Position Reagent Tube Holder with up to 3 DNase I Solution Tubes
  - Maxprep<sup>™</sup> Reagent Reservoir, 50ml with Lysis Buffer
  - Maxprep<sup>™</sup> Reagent Reservoir, 50ml with Lytic Enhancer
  - Maxprep<sup>™</sup> Reagent Reservoir, 50ml with Nuclease-Free Water
  - Maxprep<sup>™</sup> 1000µl Conductive Disposable Tips, Filtered (2; one rack may be partially full)
  - Maxprep<sup>™</sup> 300µl Conductive Disposable Tips, Filtered (rack may be partial or full)
- 8. Close the instrument door, and touch the **Next** button to start the automated preprocessing of samples.

# 5.B. Maxprep<sup>™</sup> Liquid Handler Preprocessing Protocol

The Maxprep<sup>™</sup> Liquid Handler will prepare samples prior to extraction using the Maxwell<sup>®</sup> Instruments. The following steps are performed by the Maxprep<sup>™</sup> Liquid Handler:

- 1. The system prepares a lysis reaction in the input sample tubes consisting of the following components:
  - tissue homogenate in tubes
  - 30µl of Proteinase K Solution
  - 200µl of Lysis Buffer
  - 200µl of Lytic Enhancer
- 2. The lysate incubates for 10 minutes.
- 3. During the lysis incubation, plungers are transferred to each of the cartridges in the Maxwell<sup>®</sup> deck tray(s). The specified volume of Nuclease-Free Water is transferred to the elution tubes for each position in the Maxwell<sup>®</sup> deck tray(s). Ten microliters of DNase I Solution is transferred to well# 4 of each of the cartridges in the Maxwell<sup>®</sup> deck tray(s).
- 4. After lysis incubation is complete, each sample is transferred from the 1.5ml–2.0ml tube to its corresponding Maxwell<sup>®</sup> RSC Cartridge (RSCN).

5. Method is complete. Open instrument door and move the deck tray(s) to the Maxwell<sup>®</sup> Instrument for extraction. Remove primary sample tubes and 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<sup>™</sup> 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<sup>®</sup> Instrument Setup and Run

Refer to the Operating Manual specific to your Maxwell® instrument for detailed information (see Table 1).

- 1. Turn on the Maxwell<sup>®</sup> Instrument and Tablet PC. Sign in to the Tablet PC, and start the Maxwell<sup>®</sup> 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 begin running a method.
- 3. Depending on your Maxwell<sup>®</sup> Instrument model, select a method using se one of the following options:
  - a. When running in **Portal** mode, scan the bar code(s) on the deck tray(s). After data has been returned from the Portal database, 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 **miRNA Tissue** method.
- 4. If applicable to your Maxwell<sup>®</sup> Instrument model, verify that the miRNA Tissue method has been chosen, 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 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 the 48-position Maxwell<sup>®</sup> Instruments, use the **Front** and **Back** buttons to select/deselect cartridge positions on each deck tray.



# 6. Maxwell<sup>®</sup> Instrument Setup and Run (continued)

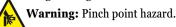
6. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with Elution Buffer and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell<sup>®</sup> 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 a Maxwell<sup>®</sup> RSC 48 Instrument, check the identifier on the Maxwell<sup>®</sup> 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 retrac t, and the door will close.

**Note:** When using the 48-position Maxwell<sup>®</sup> 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.



The Maxwell<sup>®</sup> 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 Operating Manual for your instrument (see Table 1) 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. 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.

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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. Storing Eluted RNA

If sample eluates are not processed immediately, the eluted RNA should be stored at  $-20^{\circ}$ C or  $-70^{\circ}$ C for up to 24 hours in the Maxwell<sup>®</sup> Elution Tubes. If longer term storage is desired, transfer the eluted RNA into labware that is suitable for long-term storage and store at  $-70^{\circ}$ C or below. Consult the instructions for downstream applications for specific sample storage and handling recommendations.

## 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
Sample foams during homogenization	Some homogenizers will generate foam when samples are homogenized. Allow the foam to dissipate prior to pipetting. Homogenize for shorter periods of time until visible particles and tissue fragments are eliminated. Keep rotor submerged whenever the homogenizer is on.
	Sample was homogenized in the Lysis Buffer instead of the 1-Thioglycerol/Homogenization Solution.
Homogenate is too viscous to pipet	The homogenate was too concentrated and became viscous while sitting on ice. Reduce the homogenate viscosity by increasing the amount of 1-Thioglycerol/Homogenization Solution 1.5- to 2-fold, and briefly rehomogenize the sample. The maximum volume of diluted homogenate that can be processed in a single Maxwell <sup>®</sup> RSC Cartridge (RSCN) is 200µl.
Low RNA yield, RNA degradation or poor reproducibility between samples	1-Thioglycerol was not added to the Homogenization Solution
	Lysis Buffer or Lytic Enchancer or both were not added.
	Lysates were not mixed sufficiently. Lysates must be mixed by vortexing for 20 seconds.
	For liver samples only: RNA yield for liver may be improved by incubation at 70°C for 2 minutes.



# 8. Troubleshooting (continued)

Symptoms	Causes and Comments
ow RNA yield, RNA degradation or oor reproducibility between samples continued)	Samples were not properly prepared or stored. Samples must be flash frozen, immediately homogenized in 1-Thioglycerol/Homogenization Solution or stored in RNA <i>later®</i> reagent to halt RNA degradation. Delays during sample collection may result in RNA degradation and lower yields. Freeze samples immediately and store at -70°C if they cannot be processed immediately. Homogenates should be stored at -70°C and thawed on ice.
	Homogenization was incomplete. Incomplete homogenization of samples results in loss of RNA within the particulates and clumps of debris.
	Frozen lysate was thawed by heating. Thaw frozen lysates <u>on ice or at <math>2-10^{\circ}</math>C.</u>
	RNase introduced during handling. Use sterile, disposable plasticware or baked glassware when handling RNA. Change gloves often. RNases introduced during or after purification will degrade the RNA. See Section 9, Creating a Ribonuclease-Free Environment.
	Sample contains a low amount of RNA. The amount of RNA present in a sample depends on the metabolic state, stage of growth, type of sample and growth conditions. Sample types vary in the amount of total RNA.
	The wrong method was run with the Maxwell® Instrument.
DNA contamination seen when performing RT-PCR or PCR	DNase I Solution was not added to the correct well in the cartridge, or DNase I Solution was not added at all. Check the color of the liquid in well #4. If the blue DNase I Solution was added, the reagent in well #4 will be green, not yellow.
	Too much sample was processed. Reduce the starting sample amount twofold.
	Sample has an excessive amount of genomic DNA. Reduce the starting material or increase the amount of DNase added.
	Possible cross-contamination during amplification. RT-PCR is an extremely sensitive technique. Use aerosol- resistant pipette tips. Use separate locations for pre- and post-amplification steps.



Symptoms	Causes and Comments	
DNA contamination seen when performing RT-PCR or PCR (continued)	For miRNA, too much sample was used in RT-PCR. Reduce the total RNA input to 50–100ng in RT-PCR. Generally a rare message can be detected in 50ng of total RNA by RT-PCR.	
	The wrong method was run with the Maxwell® Instrument.	
RNA purified from liver samples appears cloudy	Total RNA purified from liver may contain glycogen. When stored at 4°C or frozen, the glycogen may form a precipitate, and the sample may appear cloudy. Warm the sample to 23–25°C, and vortex to dissolve the glycogen. Glycogen does not interfere in reactions that use nucleic acids as a substrate.	
Eluate floats out of gel electrophoresis wells	Alcohol carryover in the eluate may cause it to float. Allow eluate to air-dry, or use a Speed Vac® before loading on a gel.	
Instrument is unable to pick up plungers	Use only the RSC Plungers provided in the Maxwell® RSC miRNA Tissue Kit. Plungers for Maxwell® 16 LEV kits are not compatible with the supported Maxwell® Instruments.	

# 9. Appendix

## **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, sterile, disposable plasticware should be used for handling RNA. These materials generally are 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.



#### 9. Appendix (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.

**DCaution:** 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. Related Products

#### **Instrument and Accessories**

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell <sup>®</sup> RSC Plunger Pack	48/pack	AS1670
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell <sup>®</sup> RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
Maxwell <sup>®</sup> CSC Instrument (RUO Mode only)	1 each	AS6000
Maxwell® CSC 48 Instrument (RUO Mode only)	1 each	AS8000
Maxprep <sup>™</sup> Carrier, Maxwell <sup>®</sup> RSC	1 each	AS9402
Maxprep™ Carrier, Maxwell® RSC 48 Front	1 each	AS9403
Maxprep <sup>™</sup> Carrier, Maxwell <sup>®</sup> RSC 48 Back	1 each	AS9404
Maxprep <sup>™</sup> Liquid Handler, RSC Carriers	1 each	AS9100
Maxprep™ Liquid Handler, RSC Carriers w/UV light	1 each	AS9101
Maxprep <sup>™</sup> 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
Maxprep™ Plunger Holder	1 each	AS9408
Maxprep™ 3-Position Reagent Tube Holder	1 each	AS9409

## Maxwell<sup>®</sup> RSC Reagent Kits

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

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#### 11. Summary of Changes

The following changes were made to the 3/22 revision of this document:

- 1. Updated Sections 1, 7 and 10.
- 2. Updated the cover page.

<sup>(a)</sup> U.S. Pat. No.7,329,488 and other patents.

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