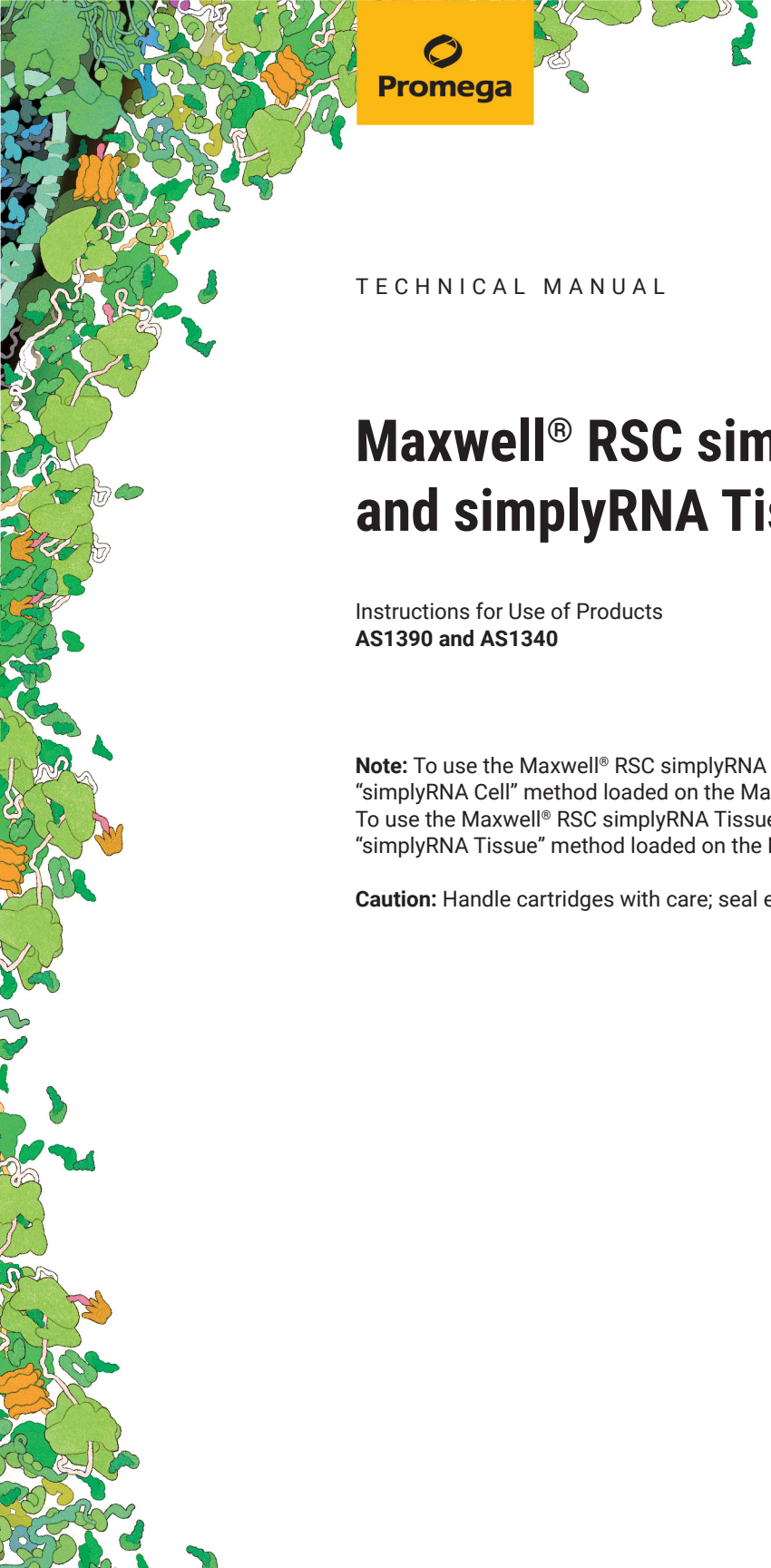


TECHNICAL MANUAL

Maxwell® RSC simplyRNA Cells and simplyRNA Tissue Kits

Instructions for Use of Products
AS1390 and AS1340

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

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

Revised 2/25
TM416

Promega

Maxwell® RSC simplyRNA Cells and simplyRNA Tissue Kits

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

Promega

1. Description

The Maxwell® RSC simplyRNA Cells Kit [(a)] (Cat.# AS1390) and Maxwell® RSC simplyRNA Tissue Kit[(a)] (Cat.# AS1340) are designed for isolation of total RNA from tissue culture cells and fresh tissue samples. The simplyRNA Cells and simplyRNA Tissue procedures purify total RNA with minimal sample handling before automated purification on the Maxwell® Instruments specified below. Maxwell® Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the RSC simplyRNA Cells Kit and the RSC simplyRNA Tissue Kit can process from one to the maximum sample number in less than 60 minutes. The low elution volume results in concentrated high-quality RNA suitable for use in downstream applications such as quantitative RT-PCR (RT-qPCR).

Table 1. Supported Instruments.

Instrument	Cat.#	Technical Manual
Maxwell® RSC	AS4500	TM411
Maxwell® RSC 48	AS8500	TM510
Maxwell® FSC	AS4600	TM462
Maxwell® CSC RUO Mode	AS6000	TM573
Maxwell® CSC 48 RUO Mode	AS8000	TM628
Maxprep® Liquid Handler	AS9100, AS9101, AS9105, AS9200, AS9201, AS9205	TM509

The Maxwell® RSC simplyRNA Cells Kit and Maxwell® RSC simplyRNA Tissue Kit purify samples using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of nucleic acid. Maxwell® 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 nucleic acids are eluted.

Before extraction, sample preprocessing can be performed manually or using the Maxprep® Liquid Handler. The Maxprep® Liquid Handler will transfer manually disrupted samples from sample tubes, perform sample lysis prior to extraction, add lysed samples to Maxwell® RSC Cartridges, transfer plungers to Maxwell® RSC 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 simplyRNA Cells Kit	**48 preps**	**AS1390**

For Research Use. Sufficient for 48 automated isolations from tissue culture cell samples. Includes:

- 30ml Homogenization Solution
- 20ml Lysis Buffer
- 900µl 1-Thioglycerol
- 1 vial DNase I (lyophilized)
- 50µl Blue Dye
- 48 Maxwell® RSC Cartridges
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes, 0.5ml
- 25ml Nuclease-Free Water

PRODUCT	SIZE	CAT.#
Maxwell® RSC simplyRNA Tissue Kit	**48 preps**	**AS1340**

For Research Use. Sufficient for 48 automated isolations from tissue samples. Includes:

- 30ml Homogenization Solution
- 20ml Lysis Buffer
- 900µl 1-Thioglycerol
- 2 vials DNase I (lyophilized)
- 50µl Blue Dye
- 48 Maxwell® RSC Cartridges
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes, 0.5ml
- 25ml Nuclease-Free Water

Storage Conditions: Upon receipt, remove 1-Thioglycerol and store at +2°C to +10°C. Store the remaining kit components at room temperature (+15°C to +30°C). 1-Thioglycerol also can be stored at room temperature (+15°C to +30°C), where it is stable for up to 9 months.

Safety Information: The Maxwell® 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.

The Maxwell® 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 used with this system.

Caution: Handle cartridges with care; seal edges may be sharp. Bleach reacts with guanidine thiocyanate and should not be added to any sample waste from these cartridges.

2. Product Components and Storage Conditions (continued)

For Preprocessing with the Maxprep® Liquid Handler

PRODUCT	SIZE	CAT.#
2.0ml Deep Well Plates (Sterile)	60/pack	AS9307
2.0ml Deep Well Plates (Non-Sterile)	60/pack	AS9309
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	48/pack	AS1670
Maxprep® Plunger Holder	1 each	AS9408

3. Sample Preparation

The Maxwell® RSC simplyRNA Cells Kit can process up to 5 × 10^6 cells. The Maxwell® RSC simplyRNA 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

Mixture of 1-Thioglycerol and Homogenization Solution

A volume of 200µl of 1-Thioglycerol/Homogenization Solution mixture is needed for each sample. To prepare a working solution, add 20µl of 1-Thioglycerol per milliliter of Homogenization Solution. 1-Thioglycerol is viscous, so careful pipetting is required for accurate measurement. Alternatively, add 600µl of 1-Thioglycerol to the 30ml bottle of Homogenization Solution. Before use, chill the mixture of 1-Thioglycerol and Homogenization Solution on ice or at +2°C to +10°C.

Note: Store the mixture of 1-Thioglycerol and Homogenization Solution at +2°C to +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 1.5ml nuclease-free tubes (such as ClickFit Microtubes, Cat.# V4741). Store reconstituted DNase I at –30°C to –10°C. DNase I solution maintains activity for up to 10 freeze-thaw cycles.

3.B. Preparation of Cell Culture Lysates

Materials to Be Supplied By the User

- centrifuge
- benchtop vortex mixer
- RNase-free, sterile, aerosol-resistant pipette tips

1. Trypsinize adherent cells following normal protocols.
2. Pellet cells at low speed (e.g., 300 × *g* for 3 minutes).
3. Remove medium.
4. Add 200µl of chilled 1-Thioglycerol/Homogenization Solution (Section 3.A) to the cell pellet and vortex until the pellet is dispersed and cells appear lysed. A pipette may be used to disperse pellets before vortexing. Alternatively, cells can be homogenized. Store lysed cells on ice if there is a delay before processing.

3.C. Preparation of Tissue Lysates

Materials to Be Supplied By the User

- small tissue homogenizer (e.g., Tissue-Tearor™ homogenizer, PRO Scientific or any homogenizer capable of handling small volumes)
- benchtop vortex mixer
- 1.5–2.0ml tube for homogenization
- RNase-free, sterile, aerosol-resistant pipette tips
- **optional:** heat block or water bath set to 70°C

1. Working as quickly as possible, homogenize the tissue sample in 200µl of chilled 1-Thioglycerol/Homogenization Solution (Section 3.A) until no visible tissue fragments remain. Homogenize an additional 15–30 seconds for complete homogenization. If foaming occurs, let sample settle on ice. Extra 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 Homogenization Solution as needed to bring samples to a final volume of 200µl.

   Note: Samples may be stored frozen at –80°C after homogenization for later processing. Thaw homogenates on ice or at 2–10°C to avoid RNA degradation.

2. **Optional:** RNA yield from larger amounts of some tissues may be increased by heating homogenates at 70°C for 2 minutes, and allowing homogenates to cool (approximately 1 minute) before proceeding. This step is recommended for 10mg or more of liver tissue.

   Note: If the heating step is used, the purified RNA may be partially denatured, and may migrate differently on native gels. Denaturing gels are recommended if the heating step is used.

Promega

4. Manual Preprocessing

Materials to Be Supplied By the User

- benchtop vortex mixer
- RNase-free, sterile, aerosol resistant pipette tips

4.A. Preprocessing of Lysates for Maxwell® RSC Cartridges

1. Prior to preparation of samples, prepare cartridges as directed in Section 4.B.
2. Add 200µl of Lysis Buffer to 200µl of cell or tissue homogenate. Vortex vigorously for 15 seconds to mix. Transfer all 400µl of lysate to well #1 (the largest well) of the Maxwell® RSC Cartridge.
3. **Cells:** Add 5µl of blue DNase I Solution (prepared as described in Section 3.A) to well #4 of the Maxwell® RSC simplyRNA Cells Cartridge (well #4 contains yellow reagent).

   Tissues: Add 10µl of blue DNase I Solution (prepared as described in Section 3.A) to well #4 of the Maxwell® RSC simplyRNA Tissue Cartridge (well #4 contains yellow reagent).

   After the blue DNase I Solution is added, the reagent in well #4 will be green.
4. Proceed to Section 6, Maxwell® Instrument Setup and Run.

4.B. Maxwell® RSC simplyRNA Cartridge Preparation

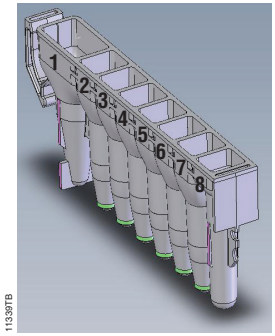
Cartridges should be prepared shortly before adding the lysate at Step 2 in Section 4.A.

1. To maintain an RNase-free environment during processing, change gloves before handling Maxwell® RSC 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 has been removed before placing cartridges in the instrument.
2. Place one plunger in 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.

Notes:

a. 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.

b. Use only the Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.



User Adds to Wells

1. Sample lysates
4. DNase I Solution
8. RSC Plunger

Figure 1. Maxwell® RSC Cartridge.

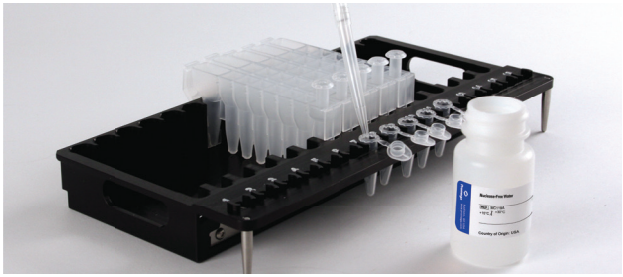


Figure 2. Setup and configuration of the deck tray(s). Nuclease-Free Water is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

5. Maxprep® Preprocessing

5.A. Maxprep® Cartridge Preparation

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, select a method using one of the two options below:
   a. Select the simplyRNA Cells or simplyRNA Tissue preprocessing method or laboratory-specific variant of the simplyRNA Cells or simplyRNA Tissue preprocessing method.
   b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate method. Select the laboratory-specific variant of the simplyRNA Cells or simplyRNA 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. Before placing Maxwell® deck tray(s) on the instrument, prepare the deck tray(s) with cartridges and elution tubes. Change gloves before handling Maxwell® RSC 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:

   a. 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.
   b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.

7. 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:

   Labware Type

   - Maxprep® Plunger Holders with Maxwell® RSC Plunger Packs (2; one may be partially full)
   - 24-position Maxwell® Front deck tray or 16-position Maxwell® deck tray containing Maxwell® RSC cartridges with seals removed and open elution tubes
   - 24-postion Maxwell® Back deck tray or 16-postion Maxwell® deck tray containing Maxwell® RSC cartridges with seals removed and open elution tubes (depending on sample number)
   - Maxprep® 3-Position Reagent Tube Holder with up to 3 tubes containing DNase I Solution
   - Maxprep® Reagent Reservoir, 50ml with Lysis Buffer
   - Maxprep® Reagent Reservoir, 50ml with Nuclease-Free Water
   - 10mm diameter tube carriers with 1.5ml or 2.0ml tubes containing Cell or Tissue lysates. 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)

8. Close the instrument door and touch the **Next** button to start the automated preprocessing of samples.

5.B. Maxprep® Liquid Handler Preprocessing Protocol

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

1. Plungers are transferred to each of the cartridges in the Maxwell® deck tray(s). The specified volume of Elution Buffer is transferred to the elution tubes for each position in the Maxwell® deck tray(s). DNase I Solution (5µl for cell lysates or 10µl for tissue lysates) is transferred to well #4 of each of the cartridges in the Maxwell® deck tray(s).
2. The system prepares a lysis reaction consisting of the following components:
   a. Cell or tissue lysate in sample tube
   b. 200µl of Lysis Buffer
3. Each sample is transferred from the sample tube to its corresponding Maxwell® RSC Cartridge.
4. Method is complete. Open instrument door and move the deck tray(s) to the Maxwell® Instrument for extraction. Remove primary sample tubes, processing plate, 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® 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

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

Table 2. Maxwell® Instrument Technical Manuals.

Instrument	Technical Manual
Maxwell® RSC	TM411
Maxwell® RSC 48	TM510
Maxwell® FSC	TM462
Maxwell® CSC RUO Mode	TM573
Maxwell® CSC 48 RUO Mode	TM628

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 begin the process of running a method.
3 Depending on your Maxwell® Instrument model, use one of the following options to select a method:
    a. When running in **Portal** mode, scan the bar code(s) on the deck tray(s). After data has been returned from the Portal software, press **Continue** to use the sample tracking information for the deck tray(s) or press **New** to start a run and enter new sample tracking information.
    b. Scan or enter the 2D bar code information on the kit box to automatically select the appropriate method.
    c. Touch the **simplyRNA Cells or simplyRNA Tissue** method.
4. If applicable to your Maxwell® Instrument model, verify that the appropriate simplyRNA method has been selected, and press the **Proceed** button. If requested by the software, scan or enter any kit lot and expiration information that has been required by the Administrator.
5. On the 'Cartridge Setup' screen (if shown), touch the cartridge positions to select or deselect the positions to be used for this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.

    Note: When using 48-position Maxwell® Instruments, press the **Front** and **Back** buttons to select and 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 Nuclease-Free Water, and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® Instrument platform.

    Inserting the Maxwell® Deck Tray(s): Hold the deck tray by the sides to avoid dislodging cartridges. Ensure that the deck tray is placed in the Maxwell® Instrument with the elution tubes closest to the door. Angle the back of the deck tray downward and place into the instrument so that the back of the deck tray is against the back of the instrument platform. Press down on the front of the deck tray to firmly seat the deck tray on the instrument platform. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation. Ensure the deck tray is level on the instrument platform and fully seated.

Note: Check the identifier on 24-position Maxwell® deck trays to determine whether they should be placed in the front or back of the instrument.

7. Touch the **Start** button to begin the extraction run. The platform will retract, and the door will close.

Warning: Pinch point hazard.

The Maxwell® Instrument will immediately begin the purification run. The screen will display information including the user who started the run, current method step being performed and approximate time remaining in the run.

Notes:

a. When using a 48-postion 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.

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

c. If a 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, perform **Clean Up** when requested.
If plungers are not present on the plunger bar, you can choose to skip **Clean Up**. The samples will be lost.

8. 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 2) to perform a **Clean Up** process to unload the plungers.

9. Remove the deck tray(s) from the instrument. Remove Elution Tubes containing RNA, and close the tubes. After the run is completed, the extraction run report will be displayed. From the 'Report View' screen, you can print or export this report or both.

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

10. Remove the cartridges and plungers from the deck tray(s), and discard as hazardous waste following your institution's recommended guidelines. Do not reuse reagent cartridges, plungers or elution tubes.

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

7. Storing Eluted RNA

If sample eluates are not processed immediately, the eluted RNA should be stored at –20°C or –70°C for up to 24 hours in the Maxwell® Elution Tubes. If longer term storage is desired, transfer the eluted RNA into RNase-free labware that is suitable for long-term storage and store at –70°C or below. Consult the instructions for downstream applications for specific sample storage and handling recommendations.

Promega

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
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 mixture.
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 mixture 1.5- to 2-fold, and briefly rehomogenize the sample. The maximum volume of homogenate that can be processed in a single Maxwell® RSC Cartridge is 200µl.
Low RNA yield, RNA degradation or poor reproducibility between samples	1-Thioglycerol was not added to the Homogenization Solution.
	Lysis Buffer was not added.
	Lysates were not mixed sufficiently. Lysates must be mixed by vortexing for 20 seconds.
	Homogenization was incomplete. Incomplete homogenization of samples results in loss of RNA within the particulates and clumps of debris.
	RNA yield for liver may be improved by incubation at 70°C for 2 minutes.
	Samples were not properly prepared or stored. Samples must be flash frozen, stored in RNA*later*® reagent or immediately homogenized in 1-Thioglycerol/Homogenization Solution mixture 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.
	Frozen lysate was heated to thaw. Thaw frozen lysates on ice or at 2–10°C.

Symptoms	Causes and Comments
Low RNA yield, RNA degradation or poor reproducibility between samples (continued)	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.
	RNase introduced by handling. Use sterile, disposable plasticware or baked glassware when handling RNA. Wear clean gloves at all times. RNases introduced during or after purification will degrade the RNA. See Section 9.A, Creating a Ribonuclease-Free Environment.
	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 no DNase I Solution was added. 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. RT-PCR and PCR are extremely sensitive techniques. Use aerosol-resistant pipette tips. Set up reactions and analyze samples in separate locations.
	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.
Purified total RNA 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.
In a gel, eluate floats out of the well when loading	Alcohol carryover in the eluate may cause it to float. Allow eluate to air dry, or use a Speed Vac® before loading in a gel.
Instrument unable to pick up plungers	Make sure you are using an RSC-specific kit; the Plungers for the Maxwell® RSC reagent kits are specific to the supported Maxwell® Instruments for this kit.

Promega

9. Appendix

9.A. 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.
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.

9.B. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® RSC Plunger Pack	1 each	AS1670
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® FSC Deck Tray	1 each	AS4016
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 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 w/UV light	1 each	AS9105
Maxprep® Liquid Handler, RSC 48 Carriers w/UV light	1 each	AS9205
2.0ml Deep Well Plates (Sterile)	60/pack	AS9307
2.0ml Deep Well Plates (Non-Sterile)	60/pack	AS9309
Maxprep® 1000µl Conductive Disposable Tips, Filtered	40/box	AS9303
Maxprep® 300µl Conductive Disposable Tips, Filtered	60/box	AS9302
Maxprep® Reagent Reservoir, 60ml	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
Maxprep® Tube Rack Stabilizer	1 each	AS1910
RNase A Solution, 4mg/ml	1ml	A7973
Cell Lysis Solution (Genomic Purification)	1,000ml	A7933
ClickFit Microtube, 1.5ml	1,000/pack	V4741

Maxwell® RSC Reagent Kits

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

10. Summary of Changes

The following changes were made to the 2/25 revision of this document:

1. Added Cat.# AS9105 and AS9205 to Table 1, Section 1. Added TM628 to Tables 1 and 2.
2. Updated fonts.
3. Updated the Maxprep trademark and the patent statement.
4. Made miscellaneous text updates, including changing Notes from numerical to alphabetical lists.
5. Updated Section 9.B, Related Products to add AS9105, AS9205 and AS1910. Removed other products.

[a]U.S. Pat. No. 6,855,499 and other patents.

Maxwell and Maxprep are registered trademarks of Promega Corporation.

RNA*later* is a registered trademark of Ambion, Inc. Speed Vac is a registered trademark of Savant Instruments, Inc. Tissue-Tearor is a trademark of BioSpec Products, Inc.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our website for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.