TECHNICAL MANUAL

Maxwell® RSC simplyRNA Cells Kit and Maxwell® RSC simplyRNA Tissue Kit

Instructions for Use of Products
AS1390 and AS1340

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

Revised 1/15
TM416

Maxwell® RSC simplyRNA Cells Kit and Maxwell® RSC simplyRNA 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® RSC simplyRNA Cells Kit[a] (Cat.# AS1390) and Maxwell® RSC simplyRNA Tissue Kit[a] (Cat.# AS1340) are used with the Maxwell® RSC Instrument (Cat.# AS4500). This RNA purification procedure is a simple method with minimal lysate handling before automated purification on the Maxwell® RSC Instrument. The low elution volume is used to generate concentrated high-quality RNA suitable for use in downstream applications such as quantitative RT-PCR. The kits provide the reagents for processing the samples and use prefilled cartridges for purification, maximizing simplicity and convenience. The Maxwell® RSC Instrument can process from 1 to 16 samples in under an hour.

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 cell samples. Includes:

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

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

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

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

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

Safety Information: The reagent cartridges contain ethanol, which is flammable. 1-Thioglycerol is toxic. Guanidine thiocyanate and guanidine hydrochloride (which are components of the Homogenization Solution and Lysis Buffer) are harmful and irritants. The Lysis Buffer also has a possible risk of harm to an unborn child. Wear gloves and follow standard safety procedures while working with these substances.

The Maxwell® RSC reagent 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.

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

3. Before You Begin

The Maxwell® RSC simplyRNA Cells Kit can process up to 5×10^6 cells. The Maxwell® RSC simplyRNA Tissue Kit can process up to 20mg of most tissues. Higher amounts of some tissues may result in higher yields (e.g., heart).

3.A. Preparation of Solutions

1-Thioglycerol/Homogenization Solution

A volume of 200µl of 1-Thioglycerol/Homogenization Solution 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 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. Store reconstituted DNase I at –30°C to –10°C. DNase I solution maintains activity for up to 10 freeze-thaw cycles.

3.B. Maxwell® RSC simplyRNA Cartridge Preparation

To maintain an RNase-free environment during processing, change gloves before handling cartridges, Maxwell® CSC/RSC Plungers and Elution Tubes. Place the cartridges to be used in the deck tray. Place each cartridge in the deck tray with the printed side 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 into the instrument.

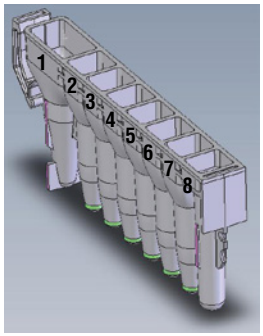
Note: If you are processing fewer than 16 samples, center the cartridges on the deck tray.

1. Place a Maxwell® CSC/RSC Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
2. Place 0.5ml Elution Tubes in the front of the deck tray. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube.

Notes:

1. If Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® RSC Instrument.
3. For setup of cell culture samples, see Section 4. For setup of tissue samples, see Section 5.

3.B. Cartridge Preparation (continued)



User Adds to Wells

1. Preprocessed samples
4. DNase I Solution
8. CSC/RSC Plunger

Figure 1. Maxwell® RSC Cartridge.

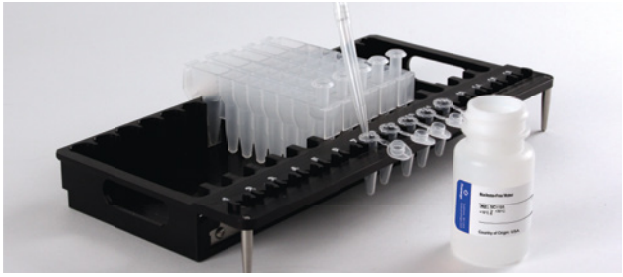


Figure 2. Setup and configuration of the deck tray. Nuclease-Free Water is added to the Elution Tubes as shown. Plungers are in well #8 of the cartridge.

4. Purification of Total RNA from Cell Culture

Materials to Be Supplied By the User

- centrifuge
- 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 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.
5. Before running the method on the Maxwell® RSC Instrument, add 200µl of Lysis Buffer (Part# MC501C) to 200µl of lysed cells. Vortex vigorously for 15 seconds to mix. Transfer all 400µl of lysate to well #1 of the Maxwell® RSC Cartridge. Well #1 is the closest to the printed side and farthest from the elution tube.
6. Add 5µl of blue DNase I Solution to well #4 (yellow reagent). After adding the blue DNase I Solution, the solution in well #4 will be green.

   Proceed to Section 6 for instructions on loading samples on the instrument and beginning the automated purification.

5. Purification of Total RNA from Tissue Samples

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)
- vortex mixer
- 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.

5. Purification of Total RNA from Tissue Samples (continued)

2. **Optional:** RNA yield from larger amounts of some tissues may be increased by heating homogenates at 70°C for 2 minutes, then allowing homogenates to cool (approximately 1 minute) before proceeding to Step 3. This is recommended for 10mg or more of liver tissue.

   Note: If the heat 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.

3. Before running the method on the Maxwell® RSC Instrument, add 200µl of Lysis Buffer (Part #MC501C) to 200µl of homogenate. Vortex vigorously for 15 seconds to mix. Transfer all 400µl of lysate to well #1 of the Maxwell® RSC Cartridge. Well #1 is closest to the printed side and farthest from the elution tube.

4. Add 5µl of blue DNase I Solution (Section 3.A) to well #4 (yellow reagent). When using more than 5mg of tissues with high DNA content (e.g., liver or spleen), add 10µl of DNase I Solution to well #4. After the blue DNase I Solution is added, the reagent in well #4 will be green.

   Proceed to Section 6 for instructions on loading samples on the instrument and beginning the automated purification.

6. Maxwell® RSC Instrument Setup and Run

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

1. Turn on the Maxwell® RSC Instrument and Tablet PC. Log on to the Tablet PC, and start the RSC software. The instrument will power up, proceed through a self-check and home all moving parts.

2. Press **Start** to access the extraction method selection screen.

3 On the extraction method selection screen, select a method using one of the two options below:

   a. Manually touch the RSC simplyRNA Cell or RSC simplyRNA Tissue 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 appropriate RSC simplyRNA method has been selected, and press the **Proceed** button. If requested by the software, enter any kit lot and expiration information that has been required by the Administrator.

5. On the cartridge selection screen, touch the cartridge positions to deselect any positions that will not be used for this extraction run. Selecting or deselecting any cartridge position is only used for reporting purposes and does not affect the way the instrument processes samples. Enter any required sample tracking information and press the **Proceed** button to continue.

6. After the door has been opened, confirm that all checklist items have been performed. Transfer the deck tray containing prepared cartridges onto the Maxwell® RSC Instrument platform. Ensure that the deck tray is placed in the Maxwell® RSC Instrument with the Elution Tubes closest to the door. If you have difficulty fitting the deck tray on the instrument platform, check that the deck tray is in the correct orientation. Ensure that the deck tray is level on the instrument platform and fully seated.

   Note: Hold the deck tray by the sides to avoid dislodging cartridges.

7. Verify that samples were added to well #1 of the cartridges, cartridges in the deck tray are loaded on the instrument, Elution Tubes are present with 50µl of Nuclease-Free Water and Maxwell® CSC/RSC Plungers are in well #8. Well #4 should be green to indicate that DNase I Solution was added.

   Note: Failure to add DNase I Solution will result in DNA in the eluate.

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

   Warning: Pinch point hazard.

   The Maxwell® RSC 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:

   1. Pressing the **Abort** button will abandon the run.
   2. If the run is abandoned before completion, the user will 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. The samples will be lost.

9. Follow on-screen instructions at the end of the method to open 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 *Maxwell® RSC Instrument Operating Manual* #TM411 to perform a Clean Up process to unload the plungers.

10. Remove the deck tray from the instrument. Remove Elution Tubes containing RNA, and close the tubes. After the run has been completed, the extraction run report will be displayed. From the report screen, you can print or export this report or both.

11. If paramagnetic particles are present in the elution tubes, centrifuge at 10,000 × *g* for 2 minutes.

    Alternatively, if desired, an additional particle capture step may be performed using the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341). Transfer the supernatant to a clean tube (not provided). Avoid transferring paramagnetic particles.

6. Maxwell® RSC Instrument Setup and Run (continued)

12. Remove the cartridges and plungers from the deck tray and discard, following your institutions recommended guidelines for disposal of hazardous material. Do not reuse reagent cartridges, plungers or Elution Tubes.

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

Storing Eluted RNA

If sample eluates are not processed immediately, the eluted RNA should be stored at –70°C, or at –20°C for up to 24 hours. Consult the protocol for your downstream application for specific storage and handling recommendations.

7. 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 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 by vortexing long enough.
	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.

Symptoms	Causes and Comments
Low RNA yield, RNA degradation or poor reproducibility between samples (continued)	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 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.
	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 8.A, Creating a Ribonuclease-Free Environment.
	The wrong method was run with the Maxwell® RSC 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 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. 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® RSC Instrument.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
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 chemistry kit; the Plungers for the Maxwell® RSC reagent kits are specific for the Maxwell® RSC Instrument.

8. Appendix

8.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.

8.B. Downstream Applications

Total RNA purified with the Maxwell® RSC simplyRNA Tissue Kit or simplyRNA Cells Kit is suitable for use in downstream applications. For more information on downstream applications, see the Promega *Protocols and Applications Guide* (1) and the *RNA Analysis Notebook* (2), available online at **www.promega.com/resources/**

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.

8.C. References

1. *Protocols and Applications Guide*, Online Edition (2011) Promega Corporation.
2. *RNA Analysis Notebook* (2002) Promega Corporation.

8.D. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC/CSC Deck Tray	1 each	SP6019

Maxwell® RSC Reagent Kits

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

Accessory Products

Product	Size	Cat.#
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	0.5ml	Z5341

9. Summary of Changes

The following change was made to the 1/15 revision of this document:

The name of the CSC Plungers was changed to CSC/RSC Plungers.

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