TECHNICAL MANUAL

Maxwell® 16 miRNA Tissue Kit

Instructions for Use of Product
AS1470

Note: To run the miRNA Method, you must have Maxwell® 16 firmware version 4.98 or higher installed on your Maxwell® 16 Instrument (Cat.# AS2000) or firmware version 1.60 or higher installed on your Maxwell® 16 MDx Instrument (Cat.# AS3000), and you must use the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070). Using the original LEV magnetic rod will result in low yields.

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

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Maxwell® 16 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® 16 miRNA Tissue Kit[a] (Cat.# AS1470) can be used with the Maxwell® 16 Instrument (Cat.# AS2000 or AS3000) to purify total RNA, including microRNA (miRNA), from tissue samples. The simple RNA purification method involves minimal sample handling before automated purification on the Maxwell® 16 Instrument. A low elution volume is used to generate concentrated high-quality RNA suitable for use in downstream applications such as quantitative RT-PCR. The Maxwell® 16 miRNA Tissue Kit provides all the reagents required for sample processing and uses prefilled cartridges for purification. The Maxwell® 16 Instrument processes 1–16 samples in about 90 minutes.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® 16 miRNA Tissue Kit	**48 preps**	**AS1470**

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

- 48 Maxwell® RSC Cartridges (RSCN)
- 30ml Homogenization Solution
- 20ml Lysis Buffer
- 20ml Lytic Enhancer
- 2 vials Proteinase K Solution
- 2 vials DNase I (lyophilized)
- 900µl 1-Thioglycerol
- 50µl Blue Dye
- 25ml Nuclease-Free Water
- 50 Maxwell® LEV 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 15–30°C, where it is stable for up to 9 months. Store rehydrated DNase I at –30°C to –10°C. Do not subject DNase I Solution to more than 10 freeze-thaw cycles.

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

The Maxwell® reagent cartridges are designed to be used with potentially infectious substances. Wear appropriate personal protective equipment (e.g., gloves and goggles) when handling infectious substances. Follow your institutional guidelines for 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

3.A. Hardware and Firmware Configuration

Firmware Requirements

The Maxwell® miRNA Tissue Kit uses the miRNA method on the Maxwell® 16 Instrument (Cat.# AS2000) and Maxwell® 16 MDx Instrument (Cat.# AS3000). To run the miRNA method you must have Maxwell® 16 firmware version 4.98 or higher installed on your Maxwell® 16 Instrument (Cat.# AS2000) or firmware version 1.60 or higher installed on your Maxwell® 16 MDx Instrument (Cat.# AS3000). Firmware is available for download at: **www.promega.com/resources/software-firmware/maxwell-maxprep/maxwell-16-system-firmware/**

Hardware Configuration

To use the Maxwell® 16 miRNA Tissue Kit, the Maxwell® 16 Instrument must be configured with the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070; Figure 1). If your Maxwell® 16 Instrument contains standard elution volume (SEV) hardware or the original Magnetic Rod, it **must be reconfigured using the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070**; Figure 1). Using the original LEV magnetic rod will result in low yields. Reconfiguring the instrument hardware is simple. Refer to the *Maxwell® 16 Instrument Operating Manual* specific for your instrument for directions.

Failure to change the Maxwell® 16 Instrument hardware to the LEV configuration could result in instrument damage. Using the standard elution volume (SEV) hardware configuration with LEV-configured reagent products will cause damage to the instrument.

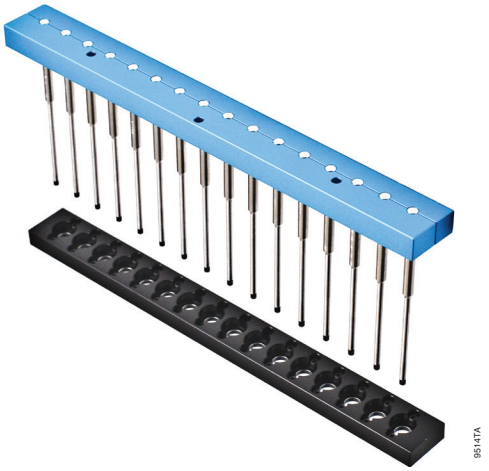


Figure 1. Maxwell 16® High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070).

3.B. 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. We recommend adding 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 any 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.C. Cartridge Preparation

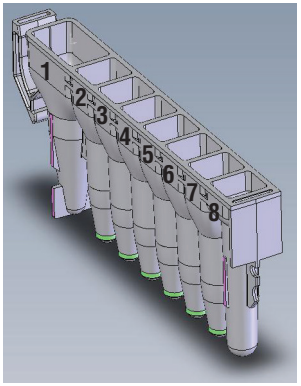
To maintain an RNase-free environment during processing, change gloves before handling cartridges, LEV Plungers and Elution Tubes. Place the cartridges to be used in the Maxwell® 16 LEV Cartridge Rack (Cat.# AS1251). Place each cartridge in the rack with the label side facing away from the Elution Tubes. 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 in the instrument.

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

1. Place an LEV 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 Maxwell® 16 LEV Cartridge Rack. Add 60µl of Nuclease-Free Water to the bottom of each Elution Tube.

Notes:

1. If you are processing fewer than 16 samples, center the cartridges on the Cartridge Rack.
2. If Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
3. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® 16 Instrument.



User Adds to Wells

1. Preprocessed samples
4. DNase I Solution
8. Maxwell® LEV Plunger

Figure 1. Maxwell® RSC Cartridge (RSCN).

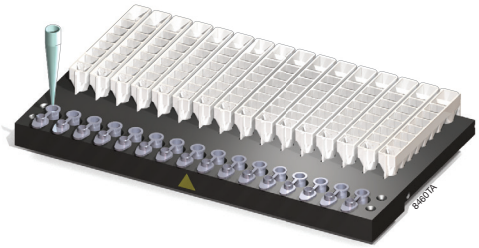


Figure 2. Setup of Elution Tubes in the Maxwell® 16 LEV Cartridge Rack. Nuclease-Free Water is added to the Elution Tubes as shown.

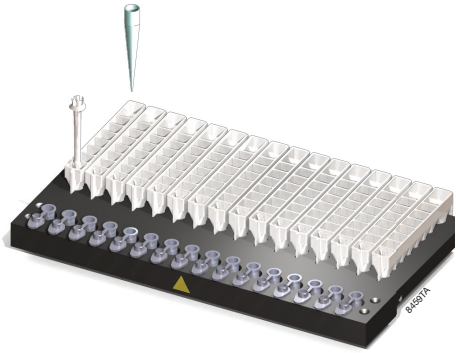


Figure 3. Setup and configuration of the Maxwell® 16 LEV Cartridge Rack. The LEV plunger is placed in well #8 of the cartridge (the well closest to the Elution Tube), and lysates are placed into well #1 of the cartridge.

4. 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

1. 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 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 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.

2. 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.

3. Incubate at room temperature for 10 minutes. During this time, prepare the Maxwell® RSC Cartridges (RSCN) as described in Section 3.C.

4. Transfer all 630µl of lysate to well #1 of the cartridge. Well #1 is the well closest to the cartridge label and farthest from the elution tube.

5. Add 10µl of blue DNase I Solution (prepared in Section 3.B) to well #4 (yellow reagent) of the cartridge. After the blue DNase I Solution is added, the reagent in well #4 will be green.

6. Proceed to Section 5 for instructions on loading samples onto the instrument and beginning the automated purification run.

5. Maxwell® 16 Instrument Setup and Run

5.A. Setup for AS2000 Maxwell® 16 Instruments

Refer to the *Maxwell® 16 Instrument Operating Manual* #TM295 for detailed information.

To run the miRNA method, you must have Maxwell® 16 firmware version **4.98** or higher installed on your instrument, and you must use the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070). Using the original LEV magnetic rod will result in low yields.

1. Turn on the Maxwell® 16 Instrument. The instrument will power up, display the firmware version number, proceed through a self-check and home all moving parts.

   Note: Ensure that you are running firmware version 4.98 or higher.

2. Verify that the instrument settings indicate "LEV" hardware configuration and "Rsch" operational mode setting.
3. Select "Run" on the Menu screen, and press the Run/Stop button to select the method.
4. Select "RNA", select "RNA" again, then select "microRNA" on the Menu screen. Next, select "OK" at the Verification screen.
5. Open the door when prompted. Press the Run/Stop button to extend the platform.

Warning: Pinch point hazard.

6. Transfer the Maxwell® 16 LEV Cartridge Rack containing prepared cartridges on the Maxwell® 16 Instrument platform. Ensure that the rack is placed in the instrument with the Elution Tubes closest to the door. The rack will only fit in the instrument in this orientation. If you have difficulty fitting the rack on the instrument platform, check that the rack is in the correct orientation. Ensure that the cartridge rack is level on the instrument platform.

   Note: Hold the Maxwell® 16 LEV Cartridge Rack by the sides to avoid dislodging cartridges from the rack.

7. Verify that samples were added to well #1 of the cartridges, cartridges in the rack are loaded on the instrument, Elution Tubes are present with 50µl of Nuclease-Free Water and LEV 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. Press the Run/Stop button. The platform will retract. Close the door.

Warning: Pinch point hazard.

5.A. Setup for AS2000 Maxwell® 16 Instruments (continued)

9. The Maxwell® 16 Instrument will immediately begin the purification run. The screen will display the steps performed and the approximate time remaining in the run.

   Notes:

   1. Pressing the Run/Stop button or opening the door will pause the run.
   2. If the run is abandoned before completion, the instrument will wash the particles off the plungers and eject the plungers into well #8 of the cartridge. To continue processing the samples, rinse any particles off the plunger into the last well used. Discard the used plungers. Put new plungers into well #8, and start the run from the beginning.

10. When the automated purification run is complete, the LCD screen will display a message that the method has ended.

End of Run

11. Follow the 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 magnetic plunger bar, push them down gently by hand to remove them.
12. Press the Run/Stop button to extend the platform out of the instrument.
13. Remove the Maxwell® 16 LEV Cartridge Rack from the instrument. Remove Elution Tubes containing miRNA, and close the tubes.
14. If paramagnetic particles are present in the elution tubes, centrifuge the eluates at 10,000 × *g* for 2 minutes. Alternatively, an additional particle capture step may be performed using the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341) or Maxwell® 16 LEV Magnet (Cat.# AS1261). Transfer the supernatant to a clean tube (not provided). Avoid transferring paramagnetic particles.
15. Remove the cartridges and plungers from the Maxwell® 16 LEV Cartridge Rack, and discard as hazardous waste according to your institution's procedures. Do not reuse reagent cartridges, LEV plungers or Elution Tubes.

Storing Eluted RNA

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

5.B. Setup for AS3000 Maxwell® 16 MDx Instruments

Refer to the *Maxwell® 16 MDx Instrument Operating Manual* #TM320 for detailed information.

To run the miRNA method, you must have Maxwell® 16 firmware version 1.60 or higher installed on your instrument, and you must use the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070). Using the original LEV magnetic rod will result in low yields.

1. Turn on the Maxwell® 16 MDx Instrument. The instrument will power up, display the firmware version number, proceed through a self-check and home all moving parts.
2. Verify that the Home screen indicates "LEV" and the LEV hardware is present. Press "Run" to continue.
3. Enter user and PIN if this option is enabled.
4. At the Protocols screen, select "RNA", then select "miRNA".
5. On the next screen, verify that the correct method and user were chosen. Select "Run/Stop" to continue.
6. Open the door when prompted on the screen, then select "Run/Stop".
7. Follow on-screen instructions for bar code reader input if this option is enabled.
8. Transfer the Maxwell® 16 LEV Cartridge Rack containing prepared cartridges on the Maxwell® 16 MDx Instrument platform. Ensure that the rack is placed in the instrument with the Elution Tubes closest to the door. The rack will only fit in the instrument in this orientation. If you have difficulty fitting the rack on the instrument platform, check that the rack is in the correct orientation. Ensure that the cartridge rack is level on the instrument platform.

   Note: Hold the Maxwell® 16 LEV Cartridge Rack by the sides to avoid dislodging cartridges.

Warning: Pinch point hazard.

9. Verify that samples were added to well #1 of the cartridges, cartridges in the rack are loaded on the instrument, Elution Tubes are present and contain 60µl of Nuclease-Free Water, and LEV 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.
10. Press the Run/Stop button. The platform will retract. Close the door.

Warning: Pinch point hazard.

The Maxwell® 16 MDx Instrument will immediately begin the purification run. The screen will display the approximate time remaining in the run.

Notes:

1. Pressing the Run/Stop button or opening the door will pause the run.
2. If the run is abandoned before completion, the instrument will wash the particles off the plungers and eject the plungers into well #8 of the cartridge. To continue processing the samples, rinse any particles off the plunger into the last well used. Discard the used plungers. Put new plungers into well #8, and start the run from the beginning.

5.B. Setup for AS3000 Maxwell® 16 MDx Instruments (continued)

11. When the automated purification run is complete, follow instructions on the screen for data transfer. For detailed instructions, refer to the *Maxwell® 16 MDx Instrument Technical Manual* #TM320 and *Maxwell® Sample Track Software Technical Manual* #TM314.

End of Run

12. Follow the 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 magnetic plunger bar, push them down gently by hand to remove them.
13. Press the Run/Stop button to extend the platform out of the instrument.
14. Remove the Maxwell® 16 LEV Cartridge Rack from the instrument. Remove Elution Tubes containing RNA, and close the tubes.
15. If paramagnetic particles are present in the elution tubes, centrifuge the eluates at 10,000 × *g* for 2 minutes. Alternatively, an additional particle capture step may be performed using the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341) or Maxwell® 16 LEV Magnet (Cat.# AS1261). Transfer the supernatant to a clean tube (not provided). Avoid transferring paramagnetic particles.

    Centrifuge samples at 10,000 × *g* for 2 minutes prior to all downstream applications.
16. Remove the cartridges and plungers from the Maxwell® 16 LEV Cartridge Rack, and discard as hazardous waste according to your institution's procedures. Do not reuse reagent cartridges, LEV plungers or Elution Tubes.

    For the Maxwell® 16 MDx Instrument, ensure samples are removed before the UV light treatment to avoid damage to the nucleic acid.

Storing Eluted RNA

If sample eluates are not processed immediately, the eluted RNA should be stored at –70°C for long-term use, or at –20°C for up to 24 hours.

6. 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® reagent cartridge 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 Enhancer or both were 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.
	For liver samples only: 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 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 thawed by heating. 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.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Low RNA yield, RNA degradation or poor reproducibility between samples (continued)	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 7.A, Creating a Ribonuclease-Free Environment.
	The wrong method was run with the Maxwell® 16 Instrument.
DNA contamination seen when performing RT-PCR	DNase I Solution was not added , or was added to an incorrect well in the cartridge. 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.
	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® 16 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 LEV Plungers provided in the Maxwell® 16 miRNA Tissue Kit. Plungers for Maxwell® RSC kits are not compatible with the Maxwell® 16 Instrument.

7. Appendix

7.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.

7.B. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® 16 Instrument*	1 each	AS2000
Maxwell® 16 MDx Instrument*	1 each	AS3000
Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor	1 each	SP1070
Maxwell® 16 LEV Magnet	1 each	AS1261

*Maxwell® 16 and Maxwell® 16 MDx Instruments have been discontinued. However, Maxwell® 16 reagent kits are still available.

Maxwell® 16 Reagent Kits

Visit **www.promega.com** for a list of available Maxwell® 16 purification kits.

Accessory Products

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

8. Summary of Changes

The following changes were made to the 11/19 revision of this document:

1. Corrected the name of the Maxwell® 16 Cartridge.
2. Updated the list of Product Components.
3. Updated the protocol in Section 4.
4. Added a new product component Lytic Enhancer.

[a]U.S. Pat. No. 7,329,488 and other patents.