TECHNICAL BULLETIN

# Maxwell® 16 Tissue LEV Total RNA Purification Kit

Instructions for Use of Product
**AS1220**

**Note:** Ensure that all sealing tape and any residual adhesive are removed from the Maxwell® 16 cartridges before placing the cartridges into the instrument.


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TB367

# Maxwell® 16 Tissue LEV Total RNA Purification 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 Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

## 1. Description

The Maxwell® 16 Tissue LEV Total RNA Purification Kit[a] is used with the Maxwell® 16 Instrument configured with the Low Elution Volume (LEV) hardware. This RNA purification procedure provides an easy method for efficient, automated purification of highly concentrated total RNA from tissue samples and whole blood collected into PAXgene® tubes. The low elution volume (30–100µl) is used to generate purified RNA in a concentrated format for use in downstream applications such as qRT-PCR, RT-PCR and cDNA synthesis. The Maxwell® 16 Instrument is supplied with preprogrammed purification procedures and is designed for use with the prefilled cartridges, maximizing simplicity and convenience. After lysate clearing, the instrument can process up to 16 samples in approximately 30 minutes.

## 2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **Maxwell® 16 Tissue LEV Total RNA Purification Kit** | **48 preps** | **AS1220** |

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

- 48 Maxwell® 16 LEV Cartridge (MCG)
- 50ml RNA Lysis Buffer (RLA)
- 44ml RNA Dilution Buffer (RDB)
- 0.9ml 97.4% β-Mercaptoethanol (BME)
- 6.5ml Clearing Agent (CAA)
- 25ml Nuclease-Free Water
- 50 Clearing Columns
- 50 Collection Tubes
- 50 LEV Plungers
- 50 Elution Tubes, 0.5ml

**Storage Conditions:** Store the Maxwell® 16 Tissue LEV Total RNA Purification Kit at 15–30°C.

**Safety Information:** The reagent cartridges contain ethanol, which is flammable, and guanidine thiocyanate, which is an irritant. β-mercaptoethanol and guanidine thiocyanate are toxic. Wear gloves and follow standard safety procedures while working with these substances.

The Maxwell® 16 reagent cartridges are designed to be used with potentially infectious substances. Wear the 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. Do not add bleach to any sample waste containing the lysate solution.

**Notes:**

1. The RNA Dilution Buffer is colored blue so that it can be easily distinguished from the other solutions.
2. Due to the toxicity of the chemicals used in the RNA purification procedure and the prevalence of RNases, wear gloves throughout the sample and cartridge preparation procedures.


| Well | Contents | User Adds: |
|---|---|---|
| 1. | Lysis Buffer | Sample |
| 2. | Paramagnetic Silica Particles | |
| 3. | Lysis Buffer | |
| 4. | Wash Buffer | |
| 5. | Wash Buffer | |
| 6. | Wash Buffer | |
| 7. | Empty | |
| 8. | Empty | Plunger |

6050TD

**Figure 1. Maxwell® 16 LEV Cartridge (MCG).**

## 3. Before You Begin

### 3.A. Maxwell® 16 Instrument Hardware Setup

The Maxwell® 16 Instrument (Cat.# AS2000) or Maxwell® 16 MDx Instrument (Cat.# AS3000) is required for use with the Maxwell® 16 Tissue LEV Total RNA Purification Kit. The Maxwell® 16 Instrument (Cat.# AS1000) must be reconfigured using the Maxwell® 16 LEV Hardware Kit (Cat.# AS1250). For instructions to properly reconfigure the AS1000 instrument, please refer to the *Maxwell® 16 Instrument Operating Manual* #TM274.

The first time that the Maxwell® 16 Instrument is powered up, a series of user prompts will appear on the Navigation LCD. Maxwell® 16 Tissue LEV Total RNA Purification Kit is intended to be used with the LEV (low elution volume) settings and the Research method on the instrument. Once the Research method is set up on the instrument, all subsequent power-ups of the instrument will automatically default to these settings.

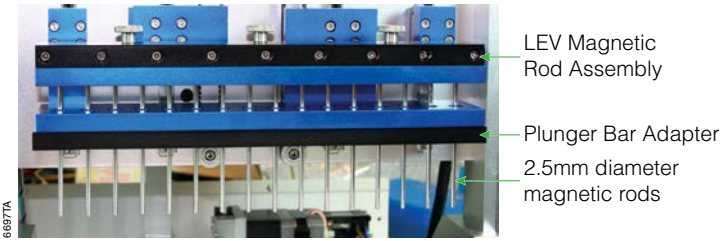


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**Figure 2. Maxwell® 16 Instrument hardware set to LEV configuration.** Refer to the *Maxwell® 16 Instrument Operating Manual* to reconfigure your instrument to the LEV hardware configuration.

### 3.A. Maxwell® 16 Instrument Hardware Setup (continued)

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

### 3.B. Maxwell® 16 Instrument Firmware Setup

The instrument firmware must be set to match the LEV hardware configuration. Also, the firmware operational mode setting must be research mode ("Rsch") for proper use of the Maxwell® 16 Tissue LEV Total RNA Purification Kit.

Failure to change the Maxwell® 16 Instrument firmware settings to LEV could result in instrument damage.

## 4. Processing Capacity of the Maxwell® 16 Tissue LEV Total RNA Purification Kit

The Maxwell® 16 Tissue LEV Total RNA Purification Kit is designed to optimize purification of concentrated high-quality total RNA for downstream applications. The capacity of the system can be exceeded by using too much sample. The processing capacity of the system depends on a variety of factors, such as sample size and type. Table 1 and Figure 3 recommend and describe the general processing capacity of the system. The specific processing capacity for your sample type may need to be determined empirically.

**Table 1. Recommended Maximum Sample Amounts.**

| Sample Type | Processing Capacity |
|---|---|
| Animal Tissue | 5–25mg |
| Stabilized Blood Sample in PAXgene® tube | 1 tube (2–2.5ml of whole blood) |

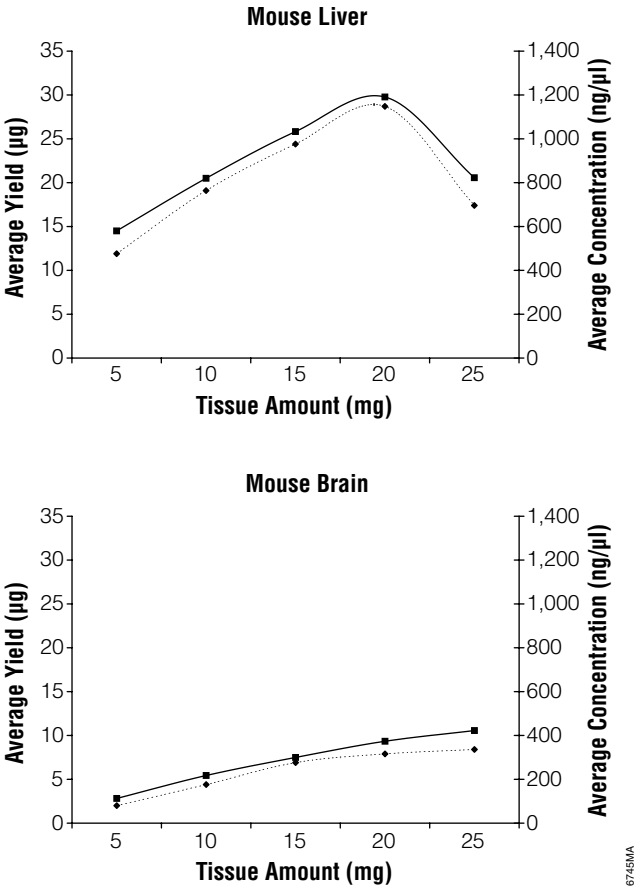


**Figure 3. Maxwell® 16 Tissue LEV Total RNA Purification Kit processing capacity will depend on sample type used.** Total RNA was purified from either 5, 10, 15, 20 or 25mg of mouse liver (a tissue with high RNA content) or mouse brain (a tissue with low RNA content) and eluted in 30µl of Nuclease-Free Water. The Average Yield (µg) of purified total RNA is plotted as a dotted line. The Average Concentration (ng/µl) of purified total RNA is plotted as a solid line.

## 4. Processing Capacity of the Maxwell® 16 Tissue LEV Total RNA Purification Kit (continued)

The elution volume of your purified sample may need to be optimized for your downstream applications. The recommended elution volume range for the Maxwell® 16 Tissue LEV Total RNA Purification Kit is 30–100µl of Nuclease-Free Water. An elution volume of 50µl is recommended. Elution volumes less than 50µl may result in lower yield. For more concentrated RNA, an elution volume of 30µl may be used, but the total yield may be affected. Do not use elution volumes less than 30µl. Elution volumes of greater than 100µl may result in purified sample too dilute for some downstream applications (Figure 4). Varying the samples sizes or elution volumes does not affect total RNA purity (Figure 5).

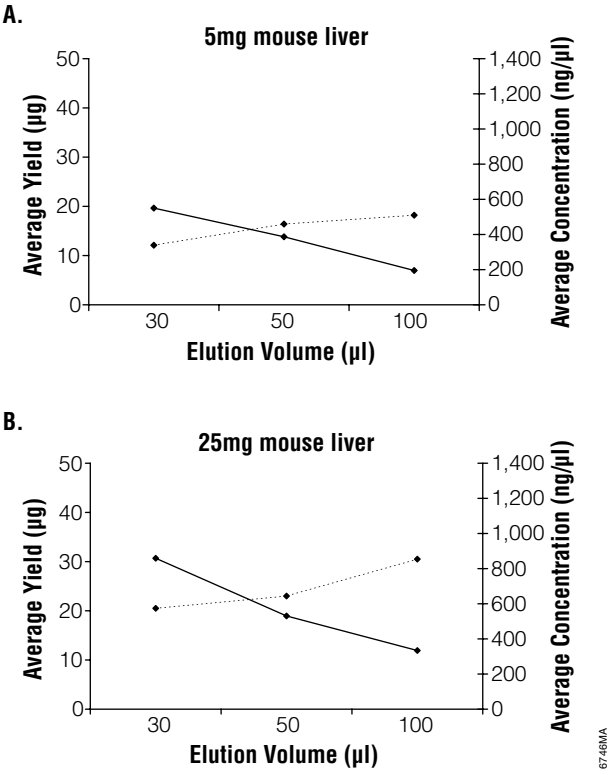


**Figure 4. Effect of different elution volumes on purified total RNA yield and concentration.** Total RNA was purified from 5 and 25mg of mouse liver and eluted in either 30, 50 or 100µl of Nuclease-Free Water. Both yield and concentration are plotted as a function of the elution volume used. The Average Yield (µg) of purified total RNA is shown as a dotted line. The Average Concentration (ng/µl) of purified total RNA is shown as a solid line. **Note:** Elution with 30µl of Nuclease-Free Water will result in the greatest final **concentration** of purified total RNA, while elution with 100µl of Nuclease-Free Water will result in a higher **yield** of purified total RNA. To maximize both yield and concentration, elute purified RNA with 50µl of Nuclease-Free Water.

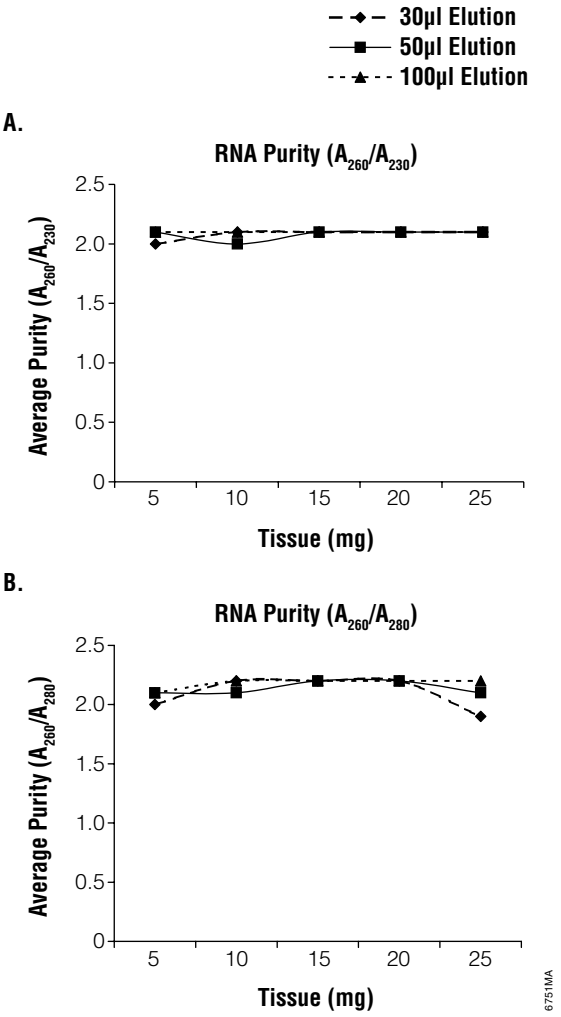


**Figure 5. Effect of different elution volumes on total purity.** Total RNA was purified from 5, 10, 15, 20 or 25mg of mouse liver and eluted in 30, 50 or 100µl of Nuclease-Free Water. The average purity of the RNA was measured using either the $A_{260}/A_{230}$ ratio or the $A_{260}/A_{280}$ ratio.

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## 5. Total RNA Isolation and Purification Procedures

Always use RNase-free pipettes and wear gloves to reduce the chance of RNase contamination.

### 5.A. Preparation of Solutions

**Materials to Be Supplied by the User**

- small tissue homogenizer (e.g., Tissue-Tearor™ homogenizer, ProScientific Model # 01-01200, or any homogenizer capable of handling small volumes)
- microcentrifuge (capable of 12,000 × *g*)
- nuclease-free polypropylene tubes
- water bath or heating block, preheated to 70°C

Prepare the RNA Lysis Buffer before beginning the Maxwell® 16 Tissue LEV Total RNA Purification Kit procedure.

Add 500µl of β-mercaptoethanol (BME) to 50ml of RNA Lysis Buffer making a final concentration of ~1% BME in the RNA Lysis Buffer. After adding the BME, mark on the bottle that this step has been completed. Alternatively, add 10µl/ml BME to single-use aliquots of RNA Lysis Buffer. Chill the RNA Lysis Buffer/BME solution on ice before use.

**Note:** Store the RNA Lysis Buffer/BME solution at 4°C, where it is stable for approximately one month. Cap the tube or bottle tightly between uses.

### 5.B. Purification of Total RNA from Tissue Samples

1. Working as quickly as possible, homogenize tissue in the Lysis Buffer/BME solution (Section 5.A). Use a maximum concentration of 125mg tissue/ml of Lysis Buffer, and homogenize until no visible tissue fragments remain. Only 200µl of the lysate can be processed per Maxwell® 16 Tissue LEV Total RNA Cartridge (Step 3). Use 1.5–5ml nuclease-free polypropylene tubes, depending on the processed volume.

   **Notes:**

   - The final volume of the lysate, regardless of how much tissue is being homogenized (up to 25mg) should be 200µl.
   - Add additional Lysis Buffer as needed to bring samples to a final volume of 200µl. Incomplete sample lysis will result in low RNA yield and purity.
   - If foaming occurs during homogenization, incubate the sample on ice until foam settles, and then rehomogenize.
   - Samples may be stored frozen after lysis for later processing; however, you may need to use less sample mass to avoid possibly clogging the Clearing Column.

2. Incubate the homogenized sample on ice for 10 minutes to ensure complete lysis.
3. Add 200µl of the homogenized sample to a 1.5–2ml tube.
4. Add 400µl of RNA Dilution Buffer. Vortex briefly to mix.
5. Vigorously shake or vortex the Clearing Agent bottle to resuspend the resin.

6. Verify that the reagent is completely resuspended by turning the bottle upside down and ensuring that there is no Clearing Agent stuck to the bottom of the reagent bottle.

   **Note:** Failure to completely resuspend the Clearing Agent may result in contaminating genomic DNA in the purified total RNA sample. The Clearing Agent settles over time and should be mixed between samples. When the Clearing Agent has been mixed properly, the pipet tip will not clog.

7. Add Clearing Agent at a volume 2 times the input tissue mass being processed according to Table 2.

   **Note:** If the lysate has been frozen, you may need to use less lysate volume to avoid the possibility of clogging the Clearing Column.

**Table 2. Volume of Clearing Agent to Add to Tissue Sample.**

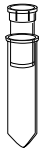
| Sample Type | Sample Tissue Mass | Volume of Clearing Agent to Add |
|---|---|---|
| Tissue Lysate (Maximum lysate concentration of 125mg/ml) | 25mg | 50µl |
| | 20mg | 40µl |
| | 15mg | 30µl |
| | 10mg | 20µl |
| | 5mg | 10µl |

8. Vortex the sample + Clearing Agent until thoroughly mixed.
9. Place the tube in a 70°C heat block for 3 minutes.

   **Note:** Ensure that the heat block is set at 70°C. (A suitable range is 65–75°C.) Heating the sample with the Clearing Agent at a lower temperature will result in incomplete clearing of genomic DNA from the sample. The heated mixtures may form clumps of precipitated debris.

10. Prepare and label a Clearing Column Assembly for each sample by placing a blue Clearing Column into a Collection Tube.
11. After heating, vortex sample + Clearing Agent briefly to resuspend the Clearing Agent.
12. Cool the sample by incubation at room temperature for 5 minutes.

    **Note:** Insufficient heating or failure to slowly cool the sample + Clearing Agent before transfer to the Clearing Column may result in genomic DNA contamination in the purified sample. Do not cool the sample on ice.



**Clearing Column Assembly (Step 10).**

13. Vortex the sample briefly, and transfer the cooled sample + Clearing Agent to the Clearing Column Assembly.
14. Centrifuge the sample at 12,000 × *g* for 2 minutes.

    **Note:** There may be a small pellet of Clearing Agent in the bottom of the collection tube. When you transfer the flowthrough to the RNA cartridge, be careful not to disturb this small pellet.

15. Proceed to Section 6.A to transfer the sample flowthrough to the cartridge.

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### 5.C. Purification of Total RNA from Whole Blood Collected into PAXgene® Tubes

PAXgene® tubes (PreAnalytix) preserve whole blood cell RNA drawn from 2.5ml of whole blood. Blood should be stored at room temperature or 4°C. Best results will be obtained after 24 hours, and up to 5 days, of storage. One Maxwell® 16 Tissue LEV Total RNA Purification cartridge will purify the RNA from one tube of white blood cells from whole blood collected into a PAXgene® tube.

**Note:** This protocol will require additional Nuclease-Free Water beyond what is supplied with this system.

1. Collect a whole blood sample into the PAXgene® tube containing stabilization solution, and mix well by inverting the tube 10 times.

   **Note:** Best results will be obtained after 24 hours, and up to 5 days, of storage at room temperature or 4°C.

2. Centrifuge the tube containing the stabilized blood solution for 10 minutes at 3,000 × *g* to pellet the white blood cells.

3. Decant the supernatant to a hazardous liquid waste container, and blot the top of the tube on a paper towel or tissue to remove any remaining solution. The cell pellet will remain on the bottom of the tube and will be brown in color from denatured hemoglobin.

4. Vortex the tube to resuspend cells in the remaining liquid then add 5ml of Nuclease-Free Water. Vortex to mix.

5. Centrifuge the tube for 10 minutes at 3,000 × *g* to pellet the white cells and decant as in Step 3. The pellet will remain brown, and the color will not affect the RNA isolation.

6. **Important:** Vigorously vortex the cell pellet in the remaining liquid until no clumps remain. Failure to mix properly will cause cell clumping and reduced RNA yield.

7. Pipet 400µl of RNA Lysis Buffer/BME solution to the bottom of the blood collection tube, mix by pipetting several times and vortex for 30 seconds. Incubate on ice for 2 minutes, and vortex again for 30 seconds until there are no visible clumps left. Transfer the lysate to a clean 1.5 or 2ml tube.

8. Using a tissue homogenizer (e.g., Tissue-Tearor™ or ProScientific model 01-01200), homogenize mixture for 10 seconds at medium speed. This is a critical step to release the RNA from the lysed cells containing genomic DNA.

   Alternatively, pass the solution through a 20-gauge needle to shear the genomic DNA. Repeat 4–5 times, ensuring that there are no clumps remaining.

   **Note:** Failure to completely lyse the sample will result in reduced yield. Continuing to Step 9 while clumps remain in the lysate will reduce RNA yield and purity.

9. Add 700µl of blue RNA Dilution Buffer, and mix by vortexing for 10 seconds.

10. Add 80µl of well-mixed Clearing Agent, and vortex for 10 seconds.

11. Incubate at 70°C for 3 minutes. Vortex tube briefly.

    **Note:** At this point the Clearing Agent should look different than it does in the reagent bottle. It will appear flocculent.

12. Cool to room temperature for a minimum of 5 minutes.

13. Prepare and label a Clearing Column for each sample by placing a blue Clearing Column into a Collection Tube.
14. Vortex and transfer 700µl of the cooled sample to the Clearing Column. The maximum volume the clearing column will hold is 700µl. This step will be repeated with the remainder of the sample.
15. Centrifuge the sample at 12,000 × *g* for 2 minutes.
16. Proceed to Section 6.A to transfer the cleared lysate to the sample well of the Maxwell® 16 LEV RNA Cartridge.
17. Transfer the remaining lysate to the same Clearing Column, and repeat Steps 15 and 16.

## 6. Automated Total RNA Purification on the Maxwell® 16 Instrument

### 6.A. Cartridge Preparation

1. Place the number of cartridges to be used into the Maxwell® 16 LEV Cartridge Rack (Cat.# AS1251). Place each cartridge into the rack with the ridged side (Figure 1) facing towards the numbered side of the rack. Slowly remove the seal. Then press down the cartridge to snap it into position.

   **Notes:**

   - Carefully peel back the plastic coating so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed from the Maxwell® 16 system cartridges before placing the cartridges into the instrument.
   - It is easiest to insert the cartridge by inserting the rigid side first, then gently pressing down on the back of the cartridge to snap it into place.
   - If you are processing fewer than 16 samples, center the reagent cartridges on the platform.


2. Place a plunger into Well #8 of each cartridge with the tabs sticking up. (Well #8 is the well closest to the elution tube).

   **Note:** The plunger will fit loosely in the cartridge.
3. Remove the blue Clearing Column from the Collection Tube.

## 6.A. Cartridge Preparation (continued)

4. Transfer the cleared lysate (flowthrough) into Well #1.
   (Well #1 is the well closest to the cartridge label and furthest from the user).

5. Place 0.5ml Elution Tubes into the elution tube holder at the front of the cartridge rack.

**Remember** to put the plunger in Well #8.

6. Add 50µl of Nuclease-Free Water to the bottom of each elution tube.

**Notes:**

1. For PAXgene® samples, we recommend using 30µl of Nuclease-Free Water.
2. If the Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.

## 6.B. Instrument Run: AS1000/AS2000 and AS3000 Instruments

### Setup for AS1000 and AS2000 Maxwell® 16 Instruments

Refer to the *Maxwell® 16 Instrument Operating Manual* #TM274 for AS1000 or #TM295 for AS2000 for more detailed information.

To run the "RNA" protocol, you must have Maxwell® 16 firmware version 4.61 or higher installed on your instrument.

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

**Warning:** Pinch point hazard.

6. Transfer the Maxwell® 16 LEV Cartridge Rack containing the prepared cartridges on the Maxwell® 16 Instrument platform. Ensure that the rack is placed in the Maxwell® 16 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 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.

8. Press the Run/Stop button. The platform will retract. Close the door.

**Warning:** Pinch point hazard.

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:**

   - Pressing the Run/Stop button or opening the door will pause the run.
   - 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. The sample will be lost.

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 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 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 cartridges and plungers from the Maxwell® 16 LEV Cartridge Rack, and discard as hazardous waste. Do not reuse reagent cartridges, LEV Plungers or Elution Tubes.

**Setup for AS3000 Maxwell® 16 MDx Instruments**

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

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”.

### 6.B. Instrument Run: AS1000/AS2000 and AS3000 Instruments (continued)

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".

**Warning:** Pinch point hazard.

7. Follow on-screen instructions for bar code reader input if this option is enabled.
8. Transfer the Maxwell® 16 LEV Cartridge Rack containing the prepared cartridges on the Maxwell® 16 Instrument platform. Ensure that the rack is placed in the Maxwell® 16 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 platform, check that the rack is in the correct orientation. Ensure the 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.
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 with 50µl of Nuclease-Free Water and LEV Plungers are in well #8.
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:**

- Pressing the Run/Stop button or opening the door will pause the run.
- 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. The samples will be lost.

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 Operating Manual* #TM320 and *Maxwell® Sample Track Software Technical Manual* #TM314.

**End of Run**

12. 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 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 cartridges and plungers from the cartridge rack, and discard as hazardous waste. 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.

## 7. Determining RNA Yield and Quality

The most common method to determine the yield and purity of RNA is spectrophotometry. The yield of total RNA is determined by measuring the sample absorbance at 260nm. According to Beer's Law, an absorbance unit ($A_{260}$ = 1.0) equals ~40µg/ml of single-stranded RNA in a 1cm pathlength cuvette. The yield of purified total RNA will vary depending on the type of starting material and physiological activity of the sample.

The $A_{260}/A_{280}$ ratio is an indicator of protein contamination. Pure RNA will exhibit an $A_{260}/A_{280}$ ratio greater than 1.8. If the sample is contaminated with protein, the ratio will be lower due to an increased absorbance or protein at 280nm.

The integrity of purified total RNA may be determined by denaturing agarose gel or Agilent Bioanalyzer analysis. RNA integrity may be reflected by the RNA integrity number (RIN) from the Agilent Bioanalyzer, which integrates several quality assessments.

## 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 |
|---|---|
| Lysate 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. |
| Lysate is too viscous to pipet | The lysate was too concentrated and became viscous while sitting on ice. Reduce lysate viscosity by increasing the amount of Lysis Solution 1.5- to 2-fold, and briefly rehomogenize the sample. The maximum volume of sample lysate that can be processed in the LEV Total RNA Purification Cartridge is 200µl. |
| Clearing Column clogged | Homogenization was incomplete. Incomplete homogenization of samples results in clumps of debris and particulates that may clog the Clearing Columns. |
| | Lysate was too concentrated. If the crude lysate is difficult to pipet, the lysate is too concentrated and may exceed the clearing capacity. Reduce the lysate viscosity by increasing the amount of Lysis Buffer. |
| | Incomplete mixing. The Cleaning Agent settles quickly in the lysate. Vortex the lysate mixture immediately before adding it to the Clearing Column. |
| | Too much sample was processed. Do not exceed recommended maximum sample amounts. For sample types that are not listed, choose the most similar sample type for guidelines. Reduce the lysate concentration twofold for samples that clog the columns. |

## 8. Troubleshooting (continued)

| Symptoms | Causes and Comments |
|---|---|
| Low RNA yield, RNA degradation or poor reproducibility between samples | The sample was not cooled before it was added to the Clearing Column. Be sure to cool the sample at room temperature before adding it to the Clearing Column. |
| | Homogenization was incomplete. Incomplete homogenization of samples results in loss of RNA within the particulates and clumps of debris. Incubate the lysate on ice for 10 minutes for complete lysis. |
| | RNA may have been degraded during sample preparation. It is essential to work quickly during sample preparation. Maintain the lysate at 4°C during preparation. |
| | BME was not added to the Lysis Buffer. |
| | Samples were not properly prepared or stored. Samples must be flash frozen or immediately homogenized in Lysis Buffer to halt RNA degradation. Delays in sample collection may result in RNA degradation and lower yields. Freeze samples immediately in liquid nitrogen, and store at –70°C if they cannot be processed immediately. Lysates should be stored at –70°C and thawed on ice. |
| | Too much Clearing Agent was used. Reduce the volume of Clearing Agent, or titrate the volume of the Clearing Agent to determine the optimal volume for the sample type. |
| | Frozen samples were thawed before homogenization. Thawing samples prior to homogenization can result in the release of RNases and RNA degradation. Keep frozen samples on dry ice until they are ready to be homogenized in Lysis Buffer. |
| | Frozen lysate was heated to thaw. Thaw frozen lysates on ice or at 4°C. To prevent RNA degradation, crude lysates should never be heated above 22–25°C. |
| | Sample contains a low abundance 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. |
| | Samples were not heated properly after adding the Clearing Agent. The heating step is critical for high RNA yields and reproducibility. Incomplete heating will result in decreased yields and decreased reproducibility. |

| Symptoms | Causes and Comments |
|---|---|
| Low RNA yield, RNA degradation or poor reproducibility between samples (continued) | 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 Maxwell® 16 Instrument was set in the wrong mode. Be sure that the instrument is set in "Rsch" mode. |
| DNA contamination seen when performing RT-PCR or PCR | Too much sample was processed. When the suggested sample amounts are used, most purified RNA samples do not show DNA contamination in RT-PCR. However, dense tissues may contain too much DNA to eliminate. Reduce the starting sample amount by twofold. |
| | Sample has an excessive amount of genomic DNA. Reduce the starting material by twofold or increase the volume of Clearing Agent. |
| | Samples were not cooled before transfer to the Clearing Column. It is important to cool the sample slowly to room temperature before adding it to the Clearing Column. |
| | 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. |
| | Clearing Agent was not used or not mixed thoroughly before use. The Clearing Agent is required for genomic DNA removal. Thorough mixing prior to dispensing ensures that the proper amount of Clearing Agent is used. To resuspend the clearing agent, shake by hand or vortex vigorously for at least 2 minutes. Then turn the reagent bottle upside down and verify that no Clearing Agent resin is stuck to the bottom of the reagent bottle. If there is, repeat vigorous shaking or vortex until no Clearing Agent resin is visible on the bottom of the reagent bottle. |
| | Samples were not mixed thoroughly. The lysate should be vortexed with the RNA Dilution Buffer as well as the Clearing Agent. |

## 8. 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. |

## 9. Appendix

### 9.A. Creating a Ribonuclease-Free Environment

Ribonucleases 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 are generally RNase-free and do not require pretreatment to inactivate RNase.
3. Treat nonsterile glassware, plasticware and electrophoresis chambers before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plasticware with 0.1N NaOH, 1mM EDTA, followed by RNase-free water. Commercially available RNase removal products may also 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.

### 9.B. Downstream Applications

Total RNA purified with the Maxwell® 16 Tissue LEV Total RNA Purification Kit is suitable for molecular biology applications such as RT-PCR, real-time qRT-PCR, cDNA synthesis and Northern blot analysis. For more information on downstream applications, see the *Protocols and Applications Guide* (1) and *RNA Applications Guide* (2).

**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.C. References


1. *Protocols and Applications Guide*, Online Edition (2004) Promega Corporation.
2. *RNA Applications Guide* (2003) Promega Corporation.


## 9.D. Related Products

| Product | Cat.# |
|---|---|
| Maxwell® 16 Instrument | AS2000 |
| Maxwell® 16 MDx Instrument | AS3000 |
| Maxwell® 16 LEV Hardware Kit | AS1250 |
| Maxwell® 16 LEV Cartridge Rack | AS1251 |
| Maxwell® 16 SEV Hardware Kit | AS1200 |
| Maxwell® 16 Cartridge Rack | AS1201 |
| Maxwell® 16 Magnetic Elution Rack | AS1202 |
| Maxwell® 16 LEV Magnet | AS1261 |

| SEV Reagent Kits | Size | Cat.# |
|---|---|---|
| Maxwell® 16 Blood DNA Purification Kit | 48 preps | AS1010 |
| Maxwell® 16 Cell DNA Purification Kit | 48 preps | AS1020 |
| Maxwell® 16 Tissue DNA Purification Kit | 48 preps | AS1030 |
| Maxwell® 16 Mouse Tail DNA Purification Kit | 48 preps | AS1120 |
| Maxwell® 16 Total RNA Purification Kit | 48 preps | AS1050 |
| Maxwell® 16 Polyhistidine Protein Purification Kit | 48 preps | AS1060 |

| LEV Reagent Kits | Size | Cat.# |
|---|---|---|
| Maxwell® 16 FFPE Tissue LEV DNA Purification Kit | 48 preps | AS1130 |
| Maxwell® 16 Tissue LEV Total RNA Purification Kit | 48 preps | AS1220 |
| Maxwell® 16 Cell LEV Total RNA Purification Kit | 48 preps | AS1225 |
| Maxwell® 16 Viral Total Nucleic Acid Purification Kit | 48 preps | AS1150 |
| Maxwell® 16 LEV Blood DNA Kit | 48 preps | AS1290 |

| Accessory Products | Size | Cat.# |
|---|---|---|
| MagneSphere® Technology Magnetic Separation Stand (twelve-position) | 1 each | Z5341 |

## 10. Summary of Changes

The following changes were made to the 7/15 revision of this document:

1. The Maxwell® 16 LEV RNA Cartridge component name was changed to Maxwell® 16 LEV Cartridge (MCG).
2. The document design was updated.