TECHNICAL BULLETIN

# Maxwell® 16 Cell LEV Total RNA Purification Kit

Instructions for Use of Product
**AS1225**

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


**Upon receipt: Store the tube of RNasin® Plus RNase Inhibitor (Cat.# AS1232) at –20°C. Store the remaining kit components (Cat.# AS1222) at room temperature (15–30°C).**

Revised 9/15
TB368

# Maxwell® 16 Cell 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 Cell 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 total RNA from the cytoplasm of eukaryotic cultured cells. The low elution volume of 30–100µl is used to generate more concentrated purified RNA for 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 pre-dispensed reagent cartridges, maximizing simplicity and convenience. After CytoSol processing, the instrument can process up to 16 samples in approximately 30 minutes.

## 2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **Maxwell® 16 Cell LEV Total RNA Purification Kit** | **48 preps** | **AS1225** |

For Laboratory Use. Cat.# AS1225 includes Cat.# AS1222 and Cat.# AS1232. Each system contains sufficient reagents for 48 purifications.

Cat.# AS1222 includes:

- 48 Maxwell® 16 LEV Cartridge (MCG)
- 50ml RNA Lysis Buffer (RLA)
- 44ml RNA Dilution Buffer (RDB)
- 0.9ml 97.4% β-Mercaptoethanol (BME)
- 10ml CytoSol Buffer
- 25ml Nuclease-Free Water
- 50 LEV Plungers
- 50 Elution Tubes (0.5ml)

Cat.# AS1232 includes:

- 0.25ml RNasin® Plus RNase Inhibitor

**Storage Conditions:** Upon receipt, remove Cat.# AS1232, RNasin® Plus RNase Inhibitor, and store at –20°C. Store the remaining kit components at room temperature (15–30°C).

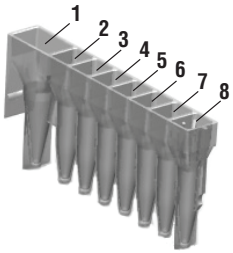
**Safety Information:** The reagent cartridges contain ethanol, which is flammable, and guanidine thiocyanate, which is an irritant. β-mercaptoethanol and guanidine thiocyanate are toxic. Wear gloves and follow standard safety procedures while working with these substances.

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. RNA Lysis Buffer (RLA) | Sample |
| 2. MagneSil® PMPs | |
| 3. RNA Lysis Buffer (RLA) | |
| 4. Yellow Core Wash Solution | |
| 5. RNA Alcohol Wash B | |
| 6. RNA Alcohol Wash B | |
| 7. RNA Alcohol Wash B | |
| 8. Empty | Plunger |
| 9. Tube holder | 0.5ml tube in tube holder slot |

6050TB

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

## 3. Before You Begin

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

To use the Maxwell® 16 Cell LEV Total RNA Purification Kit, the Maxwell® 16 Instrument must be configured with LEV instrument hardware. If your instrument contains SEV hardware, it will need to be reconfigured using the Maxwell® 16 LEV Hardware Kit (Cat.# AS1250; Figure 2). Please refer to the *Maxwell® 16 Instrument Operating Manual* for directions.

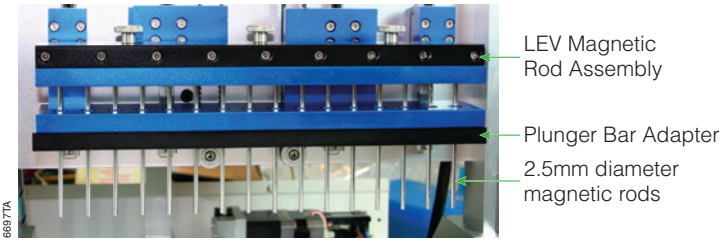



**Figure 2. Maxwell® 16 Instrument hardware set to LEV configuration.** Refer to the *Maxwell® 16 Instrument Operating Manual* to reconfigure your instrument to the LEV hardware configuration.

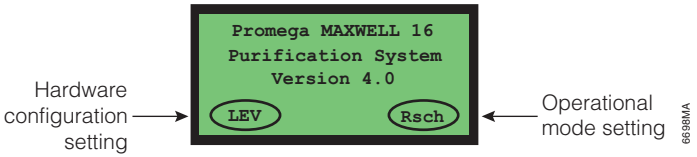
Failure to change a Maxwell® 16 Instrument's hardware to the LEV hardware configuration could result in instrument damage. Use of the standard elution volume (SEV) hardware configuration with LEV-configured reagent products will cause damage to the instrument.

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

The Maxwell® 16 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 Cell LEV Total RNA Purification Kit.

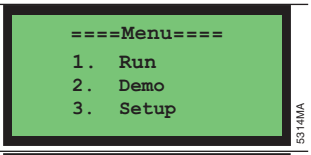
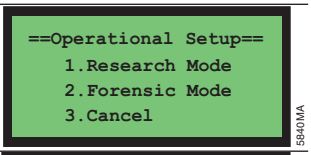
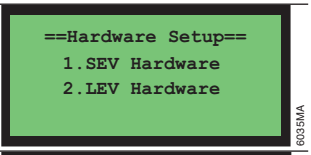
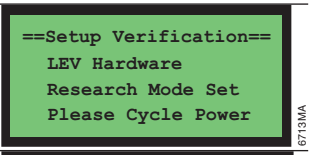
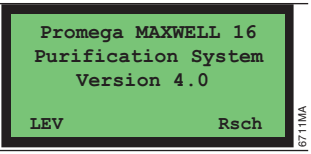
Failure to change the Maxwell® 16 Instrument firmware settings to LEV could result in instrument damage.

1. Turn the instrument off and then on again.
2. The first screen displayed on the LCD panel will show the firmware version currently installed on the instrument. Verify that firmware version 4.0 (or higher) is loaded on your instrument. If a version lower than 4.0 is loaded on your instrument, please contact Promega Technical Services for a firmware upgrade.




3. The current hardware configuration and operational mode settings are also displayed on this screen.
4. Verify that the hardware configuration setting is "LEV" and the operational mode is "Rsch".
5. If the hardware configuration and operational mode are not set to "LEV" and "Rsch", change the instrument firmware settings as indicated in Table 1.
6. Press the up/down button to make selections.

**Table 1. Changing the Maxwell® 16 Instrument Firmware Settings.**

| | |
|---|---|
| Menu screen: Select “Setup”. |  |
| Operational mode setup screen: Select “Research Mode”. |  |
| Hardware setup screen: Select “LEV Hardware”. |  |
| Confirm the instrument’s firmware settings and cycle the instrument’s power by turning the instrument off and then on again. |  |
| The correct instrument firmware setup. |  |

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

The Maxwell® 16 Cell 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 many cells. The processing capacity of the system depends on a variety of factors, such as cell size and cell number, etc. Table 2 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. For purification of stabilized white blood cells or tissue samples, please see the Maxwell® 16 Tissue LEV Total RNA Purification Kit (Cat.# AS1220).

**Table 2. Recommended Sample Amounts.**

| Sample Type | Processing Capacity |
|---|---|
| Cultured Cells | $1 \times 10^{4}$*–$2 \times 10^{6}$ cells |

*Sample sizes $1 \times 10^{4}$ cells and below may not be quantifiable by spectrophotometry but may be amplifiable by qRT-PCR.

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

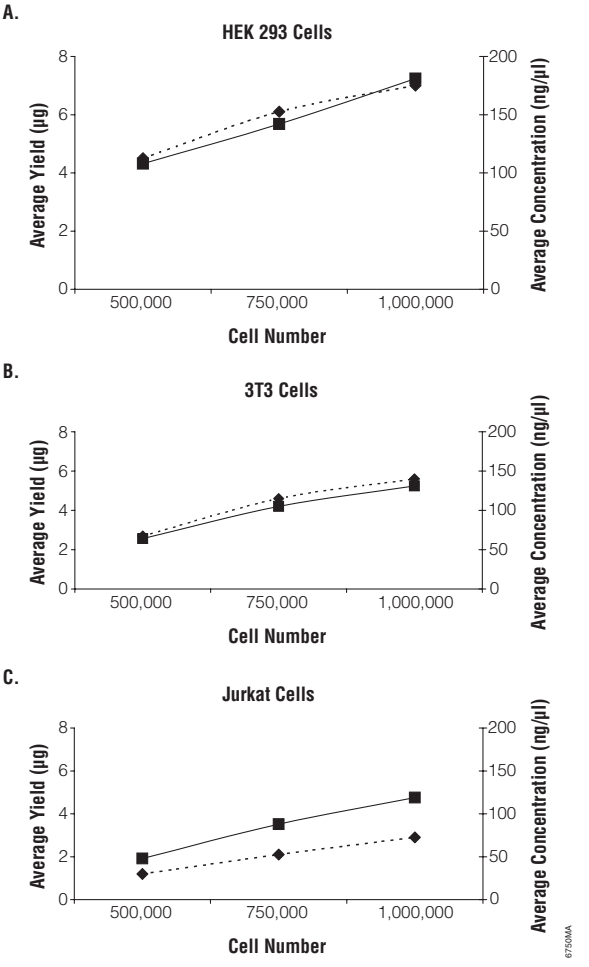



**Figure 3. Maxwell® 16 Cell LEV Total RNA Purification Kit processing capacity will depend on cell type and sample size used.** Total RNA was purified from either $5 \times 10^5$, $7.5 \times 10^5$ or $1 \times 10^6$ HEK 293, 3T3 or Jurkat cells and eluted in 50µl of Nuclease-Free Water. The Average 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. The yield and concentration of the purified total RNA depends on the starting cell type and other factors. **Panel A.** HEK 293 cells. **Panel B.** 3T3 cells. **Panel C.** Jurkat cells.

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 Cell 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 (Figure 4). Elution volumes of greater than 100µl may result in purified sample too dilute for some downstream applications. Varying the sample size or elution volume does not affect total RNA purity (Figure 5).

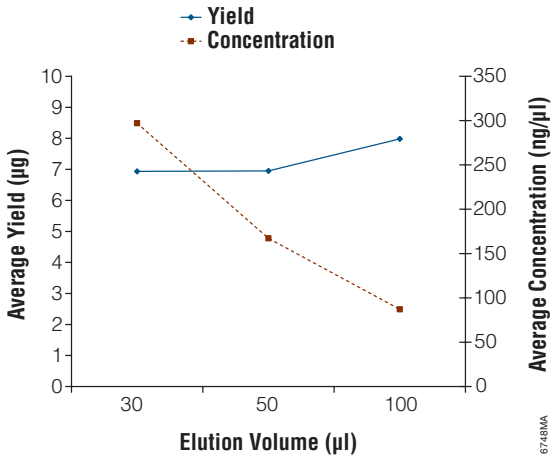



**Figure 4. Effect of different elution volumes on purified total RNA yield and concentration.** Total RNA was purified from 1 × $10^6$ HEK 293 cells and eluted in 30, 50 or 100µl of Nuclease-Free Water. The Average Yield (µg) of purified total RNA is shown as a solid line. The Average Concentration (ng/µl) of purified total RNA is shown as a dotted line. Both Yield and Concentration are plotted as a function of the elution volume used. Elution with 30µl of Nuclease-Free Water will result in the greatest **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.

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

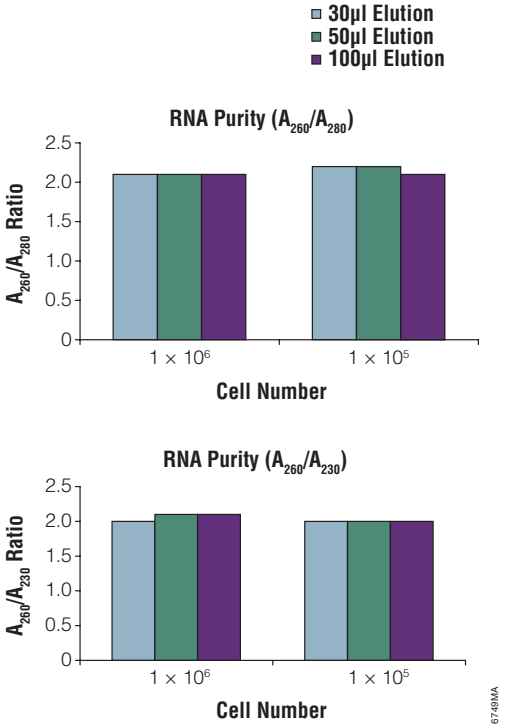



**Figure 5. Effect of different elution volumes on purified total RNA.** Total RNA was purified from $1 \times 10^6$ or $1 \times 10^5$ HEK 293 cells and eluted in 30, 50 or 100µl of Nuclease-Free Water. The average purity of the RNA was measured using either the $A_{260}/A_{280}$ ratio or the $A_{260}/A_{230}$ ratio.

## 5. RNA Isolation and Purification Procedures

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

### 5.A. Purification of Total RNA from Cultured Cells

**Materials to Be Supplied by the User**

- 1.5ml microcentrifuge tubes, nuclease free
- microcentrifuge (at 4°C) capable of 14,000 × *g*
- trypsin-EDTA for adherent cells

1. Prepare a CytoSol Working Solution (Table 3). For each sample you are processing combine in a tube 200µl of CytoSol Buffer and 2µl of RNasin® Plus RNase Inhibitor. If you are processing multiple tubes, make a master mix from which you can remove aliquots. Prepare a fresh master mix for each batch of samples.

   After preparation, keep prepared CytoSol Working Solution on ice.

**Table 3. Preparation of CytoSol Working Solution.**

| Reagent | Volume for One Sample | Volume for × N Samples |
|---|---|---|
| CytoSol Buffer | 200µl | (N + 1) × 200µl |
| RNasin® Plus RNase Inhibitor | 2µl | (N + 1) × 2µl |

2. Prepare RNA Lysis/Dilution Working Solution (Table 4). For each sample you are processing, prepare a tube with 250µl of RNA Lysis Buffer, 150µl of RNA Dilution Buffer and 4µl of β-mercaptoethanol (BME). If you are processing multiple tubes, make a master mix from which you can remove aliquots.

   The RNA Lysis/Dilution Working Solution should be made fresh for each batch of samples.

**Table 4. Preparation of RNA Lysis/Dilution Working Solution.**

| Reagent | Volume for One Sample | Volume for × N Samples |
|---|---|---|
| RNA Lysis Buffer | 250µl | (N + 1) × 250µl |
| RNA Dilution Buffer | 150µl | (N + 1) × 150µl |
| β-mercaptoethanol (BME) | 4µl | (N + 1) × 4µl |

**Prepare Cell Lysates**

3. If you are working with adherent cells, scrape or trypsinize cells to detach them from the bottom of the culture plate or flask. If you are working with suspension cells, proceed to Step 4.

**Note:** Process the cells immediately after harvesting.

4. Transfer up to 2 × $10^6$ cells into a nuclease-free tube. Pellet the cells by centrifugation at 300 × *g* for 5 minutes at 4°C. Remove the supernatant.

5. Immediately add 200µl of chilled CytoSol Working Solution (see Table 3) to the cell pellet and pipet to mix.

### 5.A. Purification of Total RNA from Cultured Cells (continued)

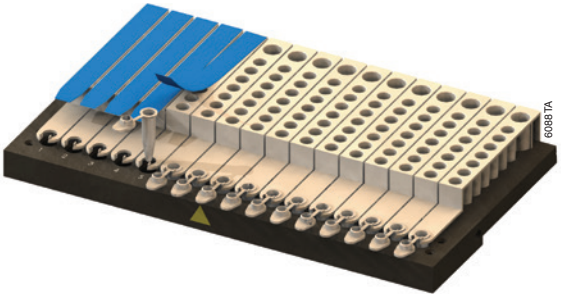
6. Vortex cells in CytoSol Working Solution for 10 seconds.
7. Incubate on ice for 10 minutes.

   **Note:** During this incubation, aliquot 400µl of the RNA Lysis/Dilution Working Solution into a new, RNase-free, 1.5ml microcentrifuge tube.
8. Centrifuge the cells + CytoSol Solution sample at the centrifuge's highest speed (at least 14,000 × *g*) for 10 minutes at 4°C.
9. After centrifugation, transfer the sample supernatant to a microcentrifuge tube containing 400µl of the RNA Lysis/Dilution Working solution.

   **Note:** Be careful to avoid the pelleted cell debris and nuclei at the bottom of the tube.
10. Vortex to mix.
11. Proceed to Section 6.A to transfer the sample to the cartridge.

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

### 6.A. Cartridge Preparation

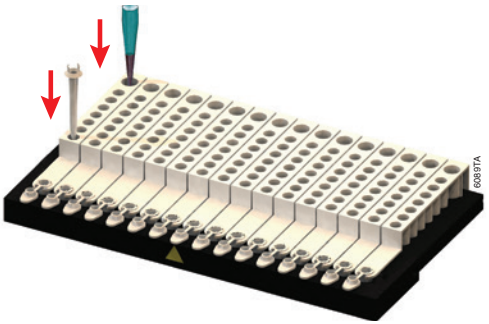



1. Place the number of cartridges to be used into the Maxwell® 16 LEV Cartridge Rack (Cat.# AS1251). Place each cartridge into the rack with the tube holder (Figure 1) facing towards the numbered side of the rack. Hold the cartridge firmly and remove the seal.

   **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 cartridges before placing the cartridges into the instrument. Alternatively, remove the seal first and then place each cartridge into the rack.
   - It is easiest to insert the cartridge by inserting the tube holder side first and 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.

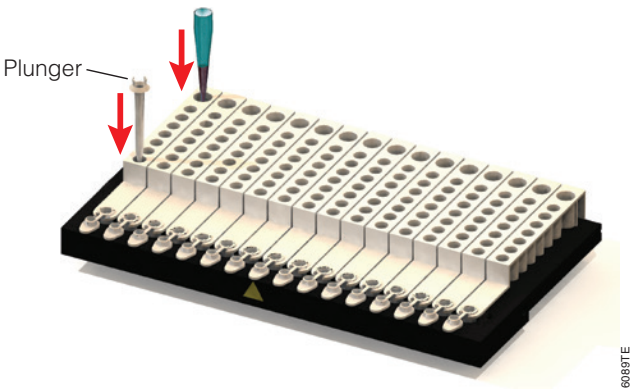
2. Place 0.5ml Elution Tubes into the elution tube holder at the front of each cartridge. Use only the Elution Tubes provided with the kit. Other 0.5ml tubes will cause errors.




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

   **Note:** The plunger will fit loosely in the cartridge.

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



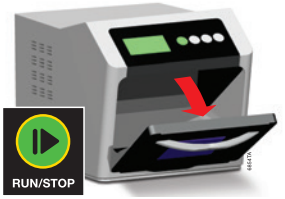

5. Add 50µl of Nuclease-Free Water to the bottom of each elution tube.

**Notes:**

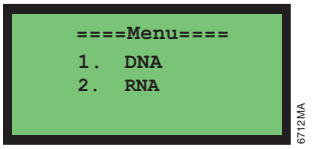
- If the Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
- Be sure the plunger is in Well #8 before beginning your run.

## 6.B. Instrument Run

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 a "Rsch" operational mode setting (Section 3.B). If the instrument firmware is not set for "LEV" and "Rsch", refer to Section 3 for information on resetting the instrument.
3. Select "Run" on the Menu screen, and press the "Run/Stop" button to start the method.




4. Select "RNA" on the Menu screen, and then select "OK" at the Verification screen.




5. Open the door when prompted to do so on the LCD display. Press the "Run/Stop" button to extend the platform.

**Warning:** Pinch point hazard.

6. Transfer the Maxwell® 16 LEV Cartridge Rack containing the prepared LEV total RNA purification cartridges onto the Maxwell® 16 Instrument platform. Ensure that the rack is placed into the Maxwell® 16 Instrument with the tube holders of the cartridges closest to the door. The rack will only fit into the instrument in this orientation. If you have difficulty fitting the rack onto the platform, check that the rack is in the correct orientation.

   **Note:** Hold the Maxwell® 16 LEV Cartridge Rack by the sides to avoid dislodging cartridges from the rack.
7. Ensure that the cartridge rack is level on the instrument platform.
8. Verify that you have added: samples to Well #1; plungers to Well #8; and Nuclease-Free Water to the 0.5ml elution tubes.
9. Press the "Run/Stop" button. The platform will retract. Close the door.

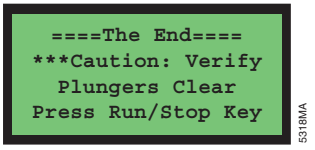
**Warning:** Pinch point hazard.

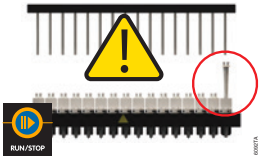
10. The Maxwell® 16 Instrument will immediately begin the purification run. The LCD screen will display the steps performed and the approximate time remaining in the run.

    **Notes:**

    - If any problems are encountered, turn the instrument off and on to restart the sequence.
    - Pressing the "Run/Stop" button or opening the door will pause the run.
    - If the program is terminated 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.

11. When the automated purification run is complete, the LCD screen will display a message that the method has ended. Upon method completion, open the instrument door. The plungers should be located in Well #8 of the cartridge at the end of the run. Check to make sure that all of the plungers have been removed from the plunger bar. If the plungers have not been removed, push them down gently by hand to remove them from the plunger bar.



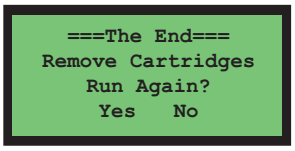

12. Press the "Run/Stop" button to extend the platform out of the instrument.

13. Remove the Elution Tubes containing eluted purified total RNA from the purification cartridges, and cap the tubes. Store the purified sample at –20°C or below for use in downstream analysis applications.

    **Notes:**

    - Some MagneSil® PMPs may be present in the elution tube. This should not affect downstream applications.
    - If desired, place the tubes containing purified total RNA sample into a magnetic elution rack (e.g., Cat.# Z5341) to do another magnetic capture of remaining MagneSil® PMPs along the side of the tube.
    - Leaving the tube in the magnetic elution rack, carefully aspirate by pipetting the purified sample to a new storage tube, being careful not to disturb any MagneSil® PMPs captured on the side of the tube.

14. Remove the cartridges and plungers from the cartridge rack and discard. Do not reuse Reagent Cartridges, Plungers, or Elution Tubes.



### 6.B. Instrument Run (continued)

15. Use the scroll up or down buttons to move the cursor to select "Yes" or "No" to run the purification method again.

    If "Yes" is selected, the Menu screen will appear.

    If "No" is selected, the platform will retract into the instrument and you will be prompted to close the instrument door.

16. A diagnostic axis check is automatically performed whether another run is chosen or not. If the check is successful, the LCD screen will display a message indicating so. If the check is unsuccessful, an error message will appear. See Section 8 for further information about resolving instrument diagnostic errors.

## 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 |
|---|---|
| Low RNA yield, RNA degradation or poor reproducibility between samples | Sample mixing may be incomplete. Incomplete permeabilization of samples may reduce RNA yield. Incubate sample on ice for 10 minutes. |
| | 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. |
| | Sample contains a low amount of RNA. The amount of RNA present in a sample depends on the metabolic state, stage of growth, cell type and growth conditions. Cell lines vary in the amount of total RNA (Figure 3). |
| | RNase was introduced by handling. Use sterile, disposable plasticware or baked glassware when handling RNA. Wear clean gloves at all times. Mishandling of kit plastics and introduction of RNases during or after the purification will degrade the RNA. See Section 9.A. |
| | RNasin® Plus was not added to the CytoSol Working Solution. RNasin® Plus inhibits RNases during sample processing. |
| | BME was not added to the RNA Lysis/Dilution Working Solution. BME inactivates RNases during sample processing. |

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## 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 Cell LEV Total RNA Purification Kit is suitable for molecular biology applications such as RT-PCR, real-time qRT-PCR, microarrays and Northern blot analysis.

**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. Related Products

| Product | Cat.# |
|---|---|
| Maxwell® 16 MDx Instrument | AS3000 |
| Maxwell® 16 LEV Hardware Kit | AS1250 |
| Maxwell® 16 SEV Hardware Kit | AS1200 |
| Maxwell® 16 LEV Cartridge Rack | AS1251 |

| Standard 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 |
| DNA IQ™ Reference Sample Kit for Maxwell® 16 | 48 preps | AS1040 |
| 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 Tissue LEV Total RNA Purification Kit | 48 preps | AS1220 |
| DNA IQ™ Casework Sample Kit for Maxwell® 16 | 48 preps | AS1210 |

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

## 9.D. Summary of Changes

The following changes were made to the 9/15 revision of this document:

1. Table 4 was updated to correct an error in the reagent listing.

[a]U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, European Pat. No. 1 204 741 and Japanese Pat. No. 4425513.