TECHNICAL MANUAL

# Maxwell® RSC miRNA Plasma and Serum Kit

Instructions for Use of Product **AS1680**


**Note:** To use the Maxwell® RSC miRNA Plasma and Serum Kit, you must have the "miRNA Plasma and Serum" method loaded on the Maxwell® Instrument.

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

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TM546

# Maxwell® RSC miRNA Plasma and Serum 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**

## 1. Description

The Maxwell® RSC miRNA Plasma and Serum Kit[a] is designed for purification of total RNA, including microRNA (miRNA), from plasma, serum or previously isolated exosomes. The miRNA Plasma and Serum procedure purifies total RNA with minimal sample handling before automated purification on the Maxwell® Instruments specified below. Maxwell® Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the Maxwell® RSC miRNA Plasma and Serum Kit can process from one to the maximum sample number in about 70 minutes. The low elution volume results in concentrated high-quality RNA suitable for use in downstream applications such as quantitative RT-PCR (RT-qPCR).

**Table 1. Supported Instruments**

| Instrument | Cat.# | Technical Manual |
|---|---|---|
| Maxwell® RSC | AS4500 | TM411 |
| Maxwell® RSC 48 | AS8500 | TM510 |
| Maxwell® FSC | AS4600 | TM462 |
| Maxwell® CSC RUO Mode | AS6000 | TM573 |
| Maxprep™ Liquid Handler | AS9100, AS9101<br>AS9200, AS9201 | TM509 |

The Maxwell® RSC miRNA Plasma and Serum Kit purifies samples using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of nucleic acid. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind nucleic acids to the paramagnetic particle in the first well of a prefilled cartridge. The samples are processed through a series of washes before the nucleic acid is eluted.

Prior to extraction, samples can be preprocessed manually or using the Maxprep™ Liquid Handler. The Maxprep™ Liquid Handler will prepare samples for preprocessing from primary tubes and can add preprocessed samples to Maxwell® RSC cartridges, transfer plungers to Maxwell® RSC cartridges and dispense elution buffer to elution tubes. Follow the instruction set specific to the preprocessing option used.

## 2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **Maxwell® RSC miRNA Plasma and Serum Kit** | **48 preps** | **AS1680** |

For Research Use Only. Not for use in diagnostic procedures. Sufficient for 48 automated isolations from plasma, serum or enriched exosome samples. Cartridges are single-use only. Includes:

- 25ml Lysis Buffer C
- 5 vials Proteinase K (PK) Solution
- 2 vials DNase I (lyophilized)
- 50µl Blue Dye
- 48 Maxwell® RSC Cartridges
- 1 RSC Plunger Pack (48 plungers)
- 50 Elution Tubes (0.5ml)
- 25ml Nuclease-Free Water

**Storage Conditions:** Store the kit components at room temperature (15–30°C). 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 Maxwell® RSC Cartridges contain ethanol and isopropanol, which are flammables and irritants. 1-Thioglycerol (which is a component of the Lysis Buffer C) is toxic. Guanidine thiocyanate (which is a component of Lysis Buffer C) is toxic, harmful and an irritant. Wear gloves and follow standard safety procedures while working with these substances. Refer to the SDS for detailed safety information. Adhere to your institutional guidelines for the handling and disposal of all chemicals and infectious substances

Maxwell® RSC Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) when handling potentialy 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 Lysis Buffer C.

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

### For Preprocessing with the Maxprep™ Liquid Handler

| PRODUCT | SIZE | CAT. # |
|---|---|---|
| **Maxprep™ 1000µl Conductive Disposable Tips, Filtered** | **40 racks of 96 tips/box** | **AS9303** |
| **Maxprep™ 300µl Conductive Disposable Tips, Filtered** | **60 racks of 96 tips/box** | **AS9302** |
| **Reagent Reservoir, 50ml** | **28/pack** | **AS9304** |
| **Nunc 2.0ml Deep Well Plates** | **60/pack** | **AS9307** |
| **Maxwell® RSC Plunger Pack** | **1 each** | **AS1670** |
| **Maxprep™ Plunger Holder** | **1 each** | **AS9408** |
| **Maxprep™ 3-Position Reagent Tube Holder** | **1 each** | **AS9409** |

## 3. Intended Use

The Maxwell® RSC miRNA Plasma and Serum Kit is intended for use in combination with supported Maxwell® Instruments with the miRNA Plasma and Serum method and is for research use only. The kit is intended for use with blood samples collected in EDTA or Serum tubes or exosomes isolated from blood or cell culture media. The Maxwell® RSC miRNA Plasma and Serum kit is not intended for use with cell culture media.

## 4. Preparing Samples and Solutions

The Maxwell® RSC miRNA Plasma and Serum Kit will produce optimal results with 100µl to 500µl of plasma or serum or 50µl to 200µl of exosomes.

### 4.A. Preparing Samples

Exosomes may be resuspended in tris buffered saline (TBS), TE or water, and 50µl to 200µl can be used in purification. Exosomes resuspended in phosphate buffered saline (PBS) perform best when less than 75µl is used for purification. Using more than 75µl of PBS may result in lower yields.

Exosomes may be resuspended in Lysis Buffer C. The total amount of Lysis Buffer C added to the cartridge, including the sample, should be 230µl. Add 200µl of Nuclease-Free Water and 80µl of proteinase K to bring the sample up to the correct volume for incubation and addition to the cartridge.

Whole blood for plasma should be processed immediately or stored at 2–10°C until plasma preparation. Whole blood for serum should be centrifuged after clotting. After plasma or serum are separated, they can be stored at 2–10°C for up to a week. For longer storage times, store plasma or serum at –30°C to –10°C (or below –65°C). Avoid multiple freeze-thaw cycles of blood products.

Exosomes/extracellular vesicles (EV) can be stored at 2–10°C for up to 3 days. For longer storage times, store EV at –30°C to –10°C (or below –65°C).

### 4.B. Preparing Solutions

#### DNase I Solution

Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I. Invert to rinse DNase off the underside of the cap and swirl gently to mix; do not vortex. Add 5µl of Blue Dye to the reconstituted DNase I as a visual aid for pipetting. Dispense the DNase I Solution into single-use aliquots in nuclease-free tubes. Each purification requires 10µl of DNase I Solution. Store reconstituted DNase I Solution at –30°C to –10°C. DNase I Solution maintains activity for up to 10 freeze-thaw cycles.

## 5. Manual Preprocessing

### 5.A. Preprocessing of Plasma or Serum Samples

1. Use plasma or serum sample volumes between 100–500µl. For plasma or serum samples less than 100µl, add 100µl of Nuclease-Free Water to the sample. Add 80µl of Proteinase K (Part# MC500C) and 230µl of Lysis Buffer C (Part# MC136A) to the plasma or serum sample. Mix by vortexing for 5 seconds.

   **Note:** Lysis Buffer C contains 1% 1-thioglycerol.

2 Incubate at 37°C for 15 minutes. During this time, prepare the Maxwell® RSC Cartridges as described in Section 5.C.

3. Transfer all of the lysate to well #1 (the largest well in the cartridge) of the Maxwell® RSC Cartridge.

4. Add 10µl of blue DNase I Solution (Section 4.A) to well #4 of the Maxwell® RSC Cartridge (well #4 contains yellow reagent). After the blue DNase I Solution is added, the reagent in well #4 will be green.

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

### 5.B. Preprocessing of Exosome Samples

**Note:** This kit does not isolate exosomes.

For optimal results, do not use more than 75µl of exosomes resuspended in PBS.

1. Up to 200µl of exosome sample in water or non-PBS buffer can be used. For exosome samples less than 200µl, add Nuclease-Free Water to the sample to bring the total volume to approximately 200µl. For exosomes suspended in Lysis Buffer C, see Section 4.A.

2. Add 80µl of Proteinase K (Part# MC500C) and 230µl of Lysis Buffer C (Part# MC136A) to the exosome sample. Mix by vortexing for 5 seconds.

3. Incubate at 37°C for 15 minutes. During this time, prepare the Maxwell® RSC Cartridges as described in Section 5.C.

4. Transfer all of the lysate to well #1 (the largest well in the cartridge) of the Maxwell® RSC Cartridge.

5. Add 10µl of blue DNase I Solution (Section 4.A) to well #4 of the Maxwell® RSC Cartridge (well #4 contains yellow reagent). After the blue DNase I Solution is added, the reagent in well #4 will be green.

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

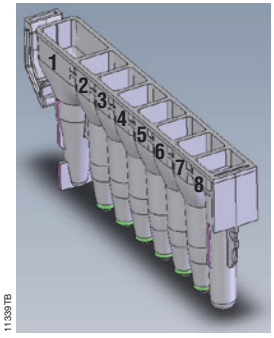
### 5.C. Maxwell® RSC miRNA Cartridge Preparation

Prepare cartridges shortly before adding the lysate at Step 3 in Section 5.A or Step 4 in Section 5.B.

1. To maintain an RNase-free environment during processing, change gloves before handling Maxwell® RSC Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
2. Place one plunger into well #8 of each cartridge.
3. Place an empty elution tube into the elution tube position for each cartridge in the deck tray. Add 50µl of Nuclease-Free Water to the bottom of each elution tube.

**Notes:**

1. Clean specimen or reagent spills on any part of the deck tray with a detergent-water solution, followed by a bacteriocidal spray or wipe and then water. Do not use bleach on any instrument parts.
2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.




**User Adds to Wells**

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

**Figure 1. Maxwell® RSC Cartridge.**

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

## 6. Maxprep™ Preprocessing

### 6.A Maxprep™ Cartridge Preparation

**Note:** Administrators must create variant methods to process exocomes and/or exosomes in lysis buffer. Options for processing exosomes and/or exosomes in lysis buffer are not available at run-time and can only be set by administrators in a variant method.

1. Turn on the Maxprep™ Liquid Handler and PC. Log in to the PC, and start the Maxprep™ software on the PC by double-clicking the desktop icon.
2. Touch **Start** to access the 'Methods' screen.
3. On the 'Methods' screen, select a method using one of the two options below:
   a. Touch the miRNA Plasma and Serum preprocessing method or laboratory-specific variant of the miRNA Plasma and Serum preprocessing method.
   b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate base method. Touch the laboratory-specific variant of the miRNA Plasma and Serum preprocessing method if desired.
4. Verify that the appropriate preprocessing method or variant method has been selected and touch the **Proceed** button. Close the instrument door and touch the **Run** button on the method run screen to start the run.
5. Enter any method-specific variables (Sample Number, Elution Volume).
6. Prior to placing Maxwell® deck tray(s) on the instrument, prepare the deck tray(s) with cartridges and elution tubes. Change gloves before handling Maxwell® RSC Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s).

   **Notes:**
   1. Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bacteriocidal spray or wipe and then water. Do not use bleach on any instrument parts.
   2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the supported Maxwell® Instruments for this kit.

### 6.A Maxprep™ Cartridge Preparation (continued)

7. Follow instrument setup instructions displayed in the method. You will be directed by the Maxprep™ software where to place the following items on the instrument:

   Maxprep™ Plunger Holders with Maxwell® RSC Plunger Packs (2; one may be partially full)

   24-position Maxwell® Front Deck Tray or 16-position Maxwell® Deck Tray containing Maxwell® RSC Cartridges with seals removed and open elution tubes

   24-position Maxwell® Back Deck Tray or 16-position Maxwell® Deck Tray containing Maxwell® RSC Cartridges with seals removed and open elution tubes

   Maxprep™ 3-Position Reagent Tube Holder with up to 3 Proteinase K Tubes (2)

   Maxprep™ 3-Position Reagent Tube Holder with up to 3 DNase I Solution Tubes

   Reagent Reservoir, 50ml with Lysis Buffer C

   Reagent Reservoir, 50ml with Nuclease-Free Water

   Maxprep™ 1000µl Conductive Disposable Tips, Filtered (2; one rack may be partially full)

   Maxprep™ 300µl Conductive Disposable Tips, Filtered (rack may be partial or full)

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

### 6.B. Maxprep™ Liquid Handler Preprocessing Protocol

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

1. The system prepares a lysis reaction in the Nunc 2.0ml Processing Plate consisting of the following components:

   a. Optional addition of Nuclease-Free Water

| Sample Type | Sample Volume | Nuclease-Free Water Volume |
|---|---|---|
| Plasma and Serum | ≤200µl | 300µl |
| Plasma and Serum | 200–500µl | Adjust Total Volume to 500µl |
| Exosomes[1] | 50–200µl | Adjust Total Volume to 200µl |
| Exosomes in Lysis Buffer C[2] | 50–200µl | 200µl |

[1]To process exosomes, create a variant method and check the **Process Exosomes (200µl Max Vol)** checkbox.
[2]To process exosomes in Lysis Buffer C, create a variant method and check the **Process Exosomes in Lysis (200µl Max Vol)** checkbox.

   b. 80µl of Proteinase K Solution

   c. 230µl of Lysis Buffer C (For exosomes in lysis buffer, Lysis Buffer C is added to adjust the total volume of Lysis Buffer C in the reaction to 230µl)

2. The Processing Plate incubates for 15 minutes.

3. During the lysis incubation the following steps are performed:
    a. Plungers are transferred to each of the cartridges in the Maxwell® deck tray(s).
    b. The specified volume of Nuclease-Free Water is transferred to the elution tubes for each position in the Maxwell® deck tray(s).
    c. 10µl of DNase I Solution is transferred to well #4 of each of the cartridges in the Maxwell® deck tray(s).
4. After lysis incubation is complete, each sample is transferred from the Processing Plate to its corresponding Maxwell® RSC cartridge.
5. Method is complete. Open instrument door and move the deck tray(s) to the Maxwell® Instrument for extraction. Remove primary sample tubes and used tips from the waste bin and discard as hazardous waste following your institution's recommended guidelines. Either discard or tightly cap and store remaining reagents

Consumables for Maxprep™ preprocessing methods are designed to be used with potentially infectious substances. Use appropriate protective equipment (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

## 7. Maxwell® Instrument Setup and Run

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

**Table 2. Maxwell® Instrument Technical Manuals**

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

1. Turn on the Maxwell® Instrument and Tablet PC. Sign in to the Tablet PC, and start the Maxwell® software by double-touching the icon on the desktop. The instrument will proceed through a self test and home all moving parts.
2. Touch **Start** to begin the process of running a method.
3. Depending on your Maxwell® Instrument model, use one of the following options to select a method:
    a. When running in Portal mode, scan the bar code(s) on the deck tray(s). After data has been returned from the Portal software, press **Continue** to use the sample tracking information for the deck tray(s) or press **New** to start a run and enter new sample tracking information.
    b. Scan or enter the 2D bar code information on the kit box to automatically select the appropriate method.
    c. Touch the **miRNA Plasma and Serum** method.

**7. Maxwell® Instrument Setup and Run (continued)**

4. If applicable to your Maxwell® Instrument model, verify that the miRNA Plasma and Serum method has been chosen, and touch the **Proceed** button. If requested by the software, scan or enter any kit lot information required by the Administrator.

5. On the 'Cartridge Setup' screen (if shown), touch the cartridge positions to select/deselect the positions to be used for this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.

   **Note:** When using a 48-position Maxwell® Instrument, touch the **Front** and **Back** buttons to select/deselect cartridge positions on each deck tray.

6. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with Elution Buffer and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® Instrument platform.

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

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

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

**Warning:** Pinch point hazard.

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

**Notes:**

1. When using a 48-position Maxwell® Instrument, if the Vision System has been enabled, the deck tray(s) will be scanned as the door retracts. Any errors in deck tray setup (e.g., plungers not in well #8, elution tubes not present and open) will cause the software to return to the 'Cartridge Setup' screen, and problem positions will be marked with an exclamation point in a red circle. Touch the exclamation point for a description of the error, and resolve all error states. Touch the **Start** button again to repeat deck tray scanning and begin the extraction run.

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

3. If the run is abandoned before completion, you may be prompted to check whether plungers are still loaded on the plunger bar. If plungers are present on the plunger bar, perform **Clean Up** when requested. If plungers are not present on the plunger bar, you can choose to skip **Clean Up** when requested. The samples will be lost.

8. 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 plunger bar, follow the instructions in the Technical Manual appropriate to your Maxwell® Instrument (see Table 2) to perform a **Clean Up** process to attempt to unload the plungers.

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

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

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

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

## 8. Storing and Quantitating RNA

If sample eluates are not processed immediately, store the eluted RNA at –20°C or –70°C. Consult the instructions for downstream applications for specific sample storage and handling recommendations.

Given the low absolute concentration of miRNA, it is not recommended to quantitate miRNA yield using absorbance methods. Quantitating by reverse transcription quantitative PCR is the most accurate way to determine quantity of miRNA in the eluate, and is recommended.

## 9. 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 | Total RNA quantitation by spectrophotometer, fluorescent dye or Bioanalyzer may not correlate with miRNA yield. Reverse transcription quantitative amplification was used to evaluate the effectivenes of miRNA purification. |
| | Lysates were not mixed sufficiently. Lysates must be mixed by vortexing for 5 seconds after reagents are added. |
| | Proteinase K Solution was not added, or incubation was not performed at correct temperature. Follow the instructions in Sections 5.A. or 5.B. |
| | Samples were not properly prepared or stored. To reduce RNA degradation, keep samples 4°C for short-term and –20°C for or –70°C long-term storage. |
| | Too little volume added to the cartridge. You may see improvements in yield with smaller sample volumes when following the recommendations on Nuclease-Free Water addition to samples in the table shown in Section 6.B, Step 1. For exosomes in lysis buffer, also confirm that the sample volume is supplemented with Lysis Buffer C to a total Lysis Buffer C volume of 230µl. |
| | Frozen sample or lysate was thawed by heating. Thaw frozen sample or lysates on ice or at 2–10°C. |
| | 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 10, Creating a Ribonuclease-Free Environment. |
| | 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. |
| | Using more than 75µl of 1xPBS in the purification may reduce miRNA recovery. Use less sample or a lower concentration of PBS. Exosomes may be resuspended in TBS or Lysis Buffer C, see Section 4.A. |
| | The wrong method was run with the Maxwell® Instrument. |

| Symptoms | Causes and Comments |
|---|---|
| DNA contamination seen when performing RT-PCR or PCR | DNase I Solution was not added to the correct well in the cartridge, or DNase I Solution was not added at all. Check the color of the liquid in well #4. If the blue DNase I Solution was added, the reagent in well #4 will be green, not yellow. |
| | Too much sample was processed. Reduce the starting sample amount twofold. |
| | Sample has an excessive amount of genomic DNA. Reduce the starting material or increase the amount of DNase added. |
| | Possible cross-contamination during amplification. RT-qPCR 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-qPCR. Follow the guidelines for miRNA input in the amplification protocol you are using. |
| | The wrong method was run with the Maxwell® Instrument. |
| Cloudy eluates | For low volume plasma and serum samples, the addition of Nuclease-Free Water to the sample during lysis can help to improve eluate clarity. Follow the recommendations on Nuclease-Free Water addition to samples in the table shown in Section 6.B, Step 1. For exosomes in lysis buffer, also confirm that the sample volume is supplemented with Lysis Buffer C to a total Lysis Buffer C volume of 230µl. |
| 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 RSC Plungers provided in the Maxwell® RSC miRNA Plasma and Serum Kit. Plungers for Maxwell® 16 LEV kits are not compatible with supported Maxwell® Instruments for this kit. |

## 10. Appendix

### 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, use sterile, disposable plasticware 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; use only in a chemical fume hood. DEPC reacts rapidly with amines and cannot be used to treat Tris buffers.

**Note:** For all downstream applications, it is essential that you continue to protect your RNA samples from RNases. Continue to wear clean gloves and use solutions and centrifuge tubes that are RNase-free.

## 11. Related Products

### Instrument and Accessories

| Product | Size | Cat.# |
|---|---|---|
| Maxwell® RSC Instrument | 1 each | AS4500 |
| Maxwell® RSC 48 Instrument | 1 each | AS8500 |
| Maxwell® FSC Instrument | 1 each | AS4600 |
| Maxwell® CSC Instrument | 1 each | AS6000 |
| Maxwell® RSC Plunger Pack | 1 each | AS1670 |
| Maxwell® RSC/CSC Deck Tray | 1 each | SP6019 |
| Maxwell® RSC 48 Front Deck Tray | 1 each | AS8401 |
| Maxwell® RSC 48 Back Deck Tray | 1 each | AS8402 |
| Maxwell® FSC Deck Tray | 1 each | AS4016 |
| Maxprep™ Carrier, Maxwell® RSC | 1 each | AS9402 |
| Maxprep™ Carrier, Maxwell® RSC 48 Front | 1 each | AS9403 |
| Maxprep™ Carrier, Maxwell® RSC 48 Back | 1 each | AS9404 |
| Maxprep™ Liquid Handler, RSC Carriers | 1 each | AS9100 |
| Maxprep™ Liquid Handler, RSC Carriers w/ UV light | 1 each | AS9101 |
| Maxprep™ Liquid Handler, RSC 48 Carriers | 1 each | AS9200 |
| Maxprep™ Liquid Handler, RSC 48 Carriers w/ UV light | 1 each | AS9201 |
| Nunc™ 2.0ml Deep Well Plates | 60/pack | AS9307 |
| Maxprep™ 1000ul Conductive Disposable Tips, Filtered | 40 racks of 96 tips/box | AS9303 |
| Maxprep™ 300ul Conductive Disposable Tips, Filtered | 60 racks of 96 tips/box | AS9302 |
| Maxprep™ Reagent Reservoir, 50ml | 28/pack | AS9304 |
| Maxprep™ Waste Bags, Clear | 100/Box | AS9305 |
| Maxprep™ Plunger Holder | 1 each | AS9408 |
| Maxprep™ 3-Position Reagent Tube Holder | 1 each | AS9409 |
| ClickFit Microtube, 1.5ml | 1,000/pack | V4741 |

### Maxwell® RSC Reagent Kits

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

## 12. Summary of Changes

The following change was made to the 9/19 revision of this document:

1. Updates were made throughout to genericize Maxwell® references for multiple supported instruments.

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



Maxwell is a registered trademark of Promega Corporation. Maxprep is a trademark of Promega Corporation.

Nunc is a trademark of Nalge Nunc International.

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

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

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