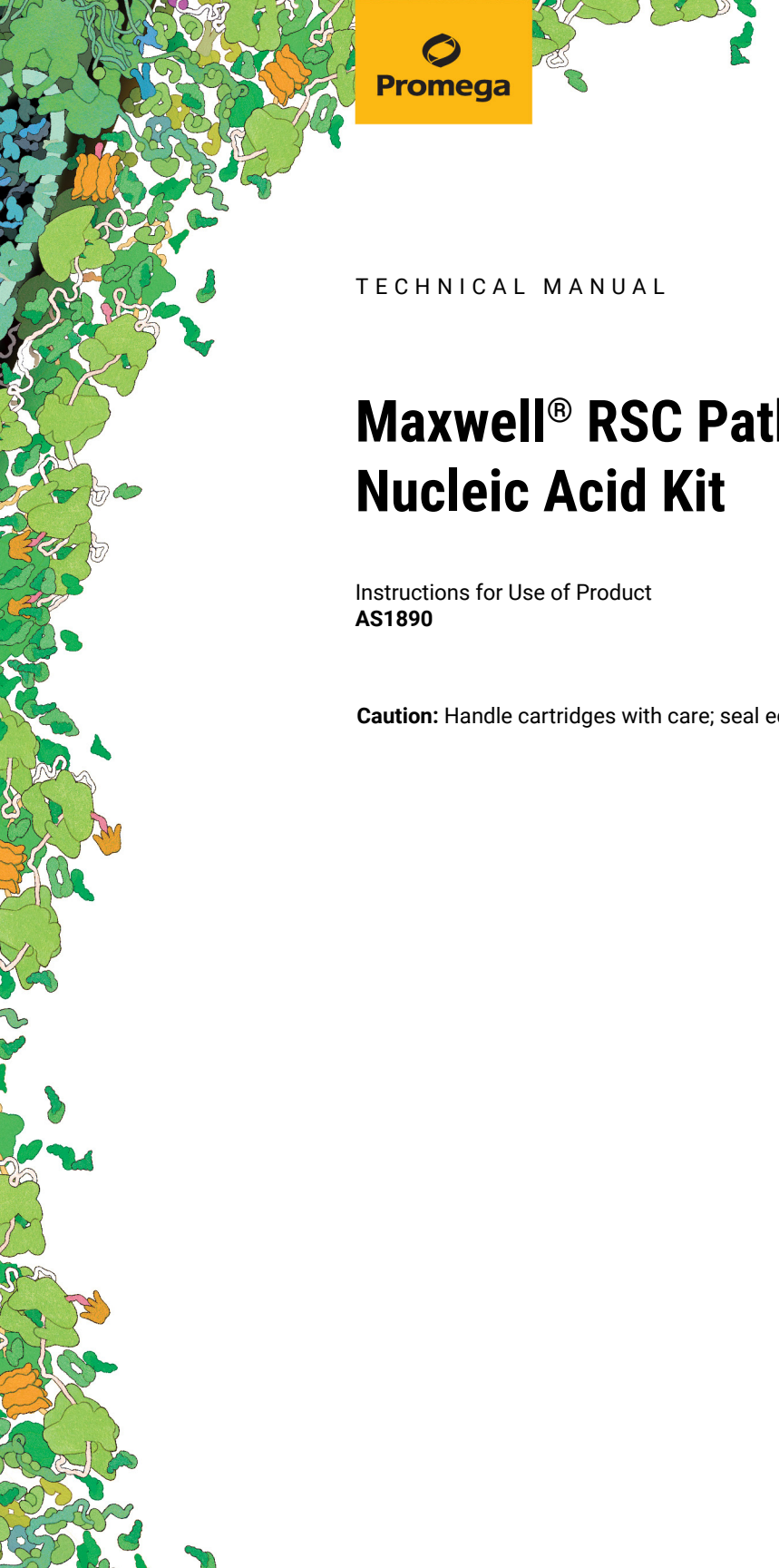

TECHNICAL MANUAL

Maxwell® RSC Pathogen Total Nucleic Acid Kit

Instructions for Use of Product
AS1890

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

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Maxwell® RSC Pathogen Total Nucleic Acid Kit

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The Maxwell® RSC Pathogen Total Nucleic Acid Kit is used with the Maxwell® Instruments specified in Table 1 to provide an easy method for efficient, automated sample preparation and purification of pathogen total nucleic acid. Maxwell® RSC Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. The Maxwell® RSC Pathogen Total Nucleic Acid Kit was designed to perform automated extraction of bacterial, viral and parasite total nucleic acid from human biological samples, including stool, sputum, bronchoalveolar lavage, urine, plasma, nasopharyngeal swabs and cervical swabs in transport medium. The Maxwell® Instrument method for the Maxwell® RSC Pathogen Total Nucleic Acid Kit can process from one to the maximum number of Maxwell® Instrument samples in approximately 40 minutes. The low 100µl elution volume results in concentrated purified nucleic acid for downstream applications, such as quantitative PCR (qPCR) or quantitative RT-PCR (RT-qPCR). After a brief preprocessing lysis step, the sample is added to the Maxwell® RSC Pathogen Total Nucleic Acid Cartridge. The remaining processing is fully automated.

Table 1. Supported Instruments.

Instrument	Cat.#	Technical Manual	Maximum Sample Number
Maxwell® RSC	AS4500	TM411	16
Maxwell® RSC 48	AS8500	TM510	48
Maxwell® FSC	AS4600	TM462	16
Maxwell® CSC RUO Mode	AS6000	TM573	16
Maxwell® CSC 48 RUO Mode	AS8000	TM628	48

Method Principle

The Maxwell® RSC Pathogen Total Nucleic Acid 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 total nucleic acid is eluted.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Pathogen Total Nucleic Acid Kit	**48 preps**	**AS1890**

For Research Use Only. Not for use in diagnostic procedures. Contains sufficient reagents for 48 automated sample isolations. Cartridges are for single use only. Includes:

- 150ml Dilution Buffer (ST1)
- 900µl 1-Thioglycerol
- 20ml Lysis Buffer
- 2 × 1ml Proteinase K (PK) Solution
- 1 Maxwell® RSC Plunger Pack (48 plungers)
- 48 Maxwell® RSC Cartridges
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Upon receipt, store 1-Thioglycerol at +2°C to +10°C. Store the remaining kit components at room temperature (+15°C to +30°C).

Safety Information: The cartridges contain ethanol, isopropanol and guanidine hydrochloride. Ethanol and isopropanol should be considered flammable, harmful and irritants. Guanidine hydrochloride should be considered toxic, harmful and an irritant. Refer to the SDS for detailed safety information.

Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

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

Additional Information: Do not use cartridges if the seal on the cartridge is not intact on receipt. For additional safety information, see the Safety Data Sheet, available at: **www.promega.com**

3. Before You Begin

Materials to Be Supplied By the User

- 1.5–2.0ml tubes for incubating samples (e.g., ClickFit Microtube, 1.5ml [Cat.# V4741]; recommended to prevent the cap from opening during heating)
- 15ml or 50ml conical tube for preparing Lysis Solution
- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- heating block or water bath set to 56°C
- heating block set to 95°C for sputum and BAL samples
- **optional:** 1X PBS to dilute BAL and sputum samples
- **optional:** centrifuge for urine sample preprocessing
- **optional:** 0.1–0.5mm zirconium beads for preprocessing of stool samples (e.g., ZR bashing beads, Zymo [Cat. #S6012-50])
- tubes for stool, sputum, bronchoalveolar lavage (BAL), viral transport medium (VTM), Pap medium, plasma and urine samples

Blood-borne pathogen precautions are recommended when handling any human-derived specimens.

For plasma samples, collect blood in EDTA-anticoagulant tubes. Avoid using heparin-containing blood collection tubes because heparin can inhibit downstream amplifications.

The following general recommendations are for preparing and storing samples (from references 1–4):

1. Separate plasma from cells within 1 hour of drawing blood by centrifuging at 1,500 × *g* for 20 minutes at 25°C, and then transfer plasma layer into a clean tube. Store plasma at 2–8°C for up to 24 hours, or freeze samples that are not processed within 24 hours at –20°C for up to 5 days.
2. For swabs in VTM, use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inhibit PCR testing. Place swabs immediately into sterile tubes containing 2–3ml of viral transport medium. Store VTM and samples at 2–8°C for up to 72 hours, or freeze samples at –70°C. Avoid repeated freeze-thaw cycles, and do not store samples in a frost-free freezer. Specific collection and storage conditions may vary, depending on the virus isolated.
3. Store fresh urine samples at 4°C for up to 24 hours before processing. For longer term storage, add EDTA to a final concentration of 20mM. To avoid cell lysis, store stabilized urine at 4°C. To avoid cell lysis, urine should not be frozen. If urine is frozen and thawed, a precipitate may become visible. Mixing and/or heating of the sample should resolubilize the precipitate. Avoid centrifuging the sample to remove precipitate because this can remove intact pathogenic cells.
4. Store BAL and sputum samples at 2–8°C for up to 72 hours, or freeze samples at –70°C. Avoid repeated freeze-thaw cycles, and do not store samples in a frost-free freezer. Specific collection and storage conditions may vary, depending on the pathogen isolated.
5. Stool samples should be frozen at –20°C to –80°C before processing.

4. Sample Preparation for Use with the Maxwell® RSC Pathogen Total Nucleic Acid Kit

4.A. Preparing Stool Samples

1. Weigh 100–500mg of fecal sample into a 2ml screw-cap microcentrifuge tube.
2. Add 1ml of Dilution Buffer (ST1) to resuspend sample. Vortex vigorously for 30 seconds to 1 minute. Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
3. Centrifuge sample 1,000 × *g* for 1 minute at room temperature.
4. Transfer supernatant to appropriately sized tube and measure volume.
5. Add 2X volume of Dilution Buffer (ST1) to stool supernatant [e.g., 1ml of stool supernatant + 2ml of Dilution Buffer (ST1)]. Use this stool sample dilution for DNA purifications.

   Note: For extracting viral RNA, further dilute the stool sample with 7X volume of nuclease-free water (e.g., 100µl of diluted stool sample from Step 5 + 700µl of nuclease-free water). Store the sample at room temperature until ready for processing.

   Optional Bead Beating Method for Extracting DNA from Difficult-to-Lyse Pathogens in Stool Samples

   Note: For extracting nucleic acids from difficult-to-lyse pathogens such as Gram-positive bacteria or protozoa in stool specimens, an optional bead-beating step can be performed. This additional homogenization can increase nucleic acid purification yield if the standard lysis method is insufficient.

   a. Transfer 600µl to 1ml of diluted stool sample to a 2ml tube containing beads and close tube.
   b. Place tubes on plate vortexer or equivalent and vortex or bead beat at maximum speed for 10 minutes.
   c. Centrifuge tubes in a microcentrifuge for 1 minute at full speed to separate beads from sample.
6. Add 300µl of stool lysate to new 1.5ml tube.
7. Add 300µl of Lysis Buffer and mix by vortex for 10 seconds.
8. Add 30µl of Proteinase K Solution and vortex briefly to mix.
9. Incubate at 56°C for 20 minutes.
10. Proceed to Maxwell® RSC Pathogen Total Nucleic Acid cartridge preparation (Section 4.E).

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4.B. Preparing BAL or Sputum Samples

1. If frozen, thaw BAL or sputum sample at room temperature.

   Note: To reduce viscosity of BAL and sputum samples, dilute in an equal volume of 1X PBS, mix by pipetting using a wide-bore pipette tip, or dilute and homogenize prior to processing.

2. For each sample, add 100–300µl to a 1.5ml microcentrifuge tube. (We recommend using a wide-bore pipette). Use fresh pipette tips for each sample to prevent sample-to-sample contamination.

3. Add 12µl of 1-Thioglycerol and pipet to mix.

   Note: Pipet slowly because the solution is viscous.

4. Add 300µl of Lysis Buffer and mix by vortexing for 10 seconds.

5. Incubate at 95°C for 5 minutes. Cool on the benchtop for 2 minutes.

6. Add 30µl of Proteinase K Solution and vortex briefly to mix.

7. Incubate at 56°C for 20 minutes.

8. Proceed to Maxwell® RSC Pathogen Total Nucleic Acid cartridge preparation (Section 4.E).

4.C. Preparing Urine Samples

Preparing small sample volumes (300µl of urine):

1. Vortex urine sample briefly to resuspend any settled cells.
2. For each sample, add 300µl of urine to a 1.5ml microcentrifuge tube. Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
3. Add 300µl of Lysis Buffer and mix by vortexing for 10 seconds.
4. Add 30µl of Proteinase K Solution and vortex briefly to mix.
5. Incubate at 56°C for 20 minutes.
6. Proceed to Maxwell® RSC Pathogen Total Nucleic Acid cartridge preparation (Section 4.E).

Preparing large sample volumes (30ml of urine):

1. Vortex urine sample briefly to resuspend any settled cells.
2. For each sample, add 30ml of urine to a 50ml tube. Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
3. Centrifuge urine sample at 2,000 × *g* for 10 minutes.
4. Remove supernatant with a pipette, being careful not to disturb the pellet.
   Note: Some liquid can be left behind to preserve pellet integrity (up to 300µl).
5. Add 300µl of Lysis Buffer to tube and resuspend pellet by pipetting.
6. Transfer the lysate to a 1.5ml tube.
7. Add 30µl of Proteinase K Solution and vortex briefly to mix.
8. Incubate at 56°C for 20 minutes.
9. Proceed to Maxwell® RSC Pathogen Total Nucleic Acid cartridge preparation (Section 4.E).

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4.D. Preparing Plasma, Viral Transport Media or Pap Media Samples

1. If frozen, thaw plasma or media at room temperature and vortex briefly to mix.
2. For each sample, add 300µl of plasma or medium containing biological sample to a 1.5ml microcentrifuge tube. Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
3. Add 300µl of Lysis Buffer and mix by vortexing for 10 seconds.
4. Add 30µl of Proteinase K Solution and vortex briefly to mix.
5. Incubate at 56°C for 20 minutes.
6. Proceed to Maxwell® RSC Pathogen Total Nucleic Acid cartridge preparation (Section 4.E).

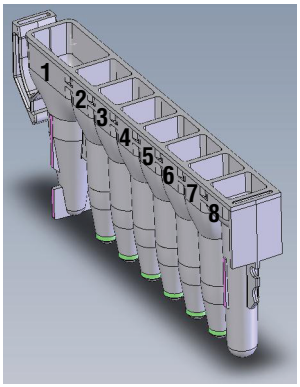
4.E. Preparing the Maxwell® RSC Pathogen Total Nucleic Acid Cartridges

1. Change gloves before handling cartridges, 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 is removed from 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 (0.5ml) into the elution tube position for each cartridge in the deck tray(s).
4. Add 100µl of Elution Buffer to the bottom of each Elution Tube.
5. Briefly spin sample lysates in a microcentrifuge to collect liquid at the bottom of the tube. Transfer sample lysate to well #1 (the largest well) of the cartridge. Do not add anything other than the sample lysate to the cartridge.
6. Proceed to Section 5, Maxwell® Instrument Run.

   Notes:

   a. Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bactericidal spray or wipe and then water. Do not use bleach on instrument parts.

   b. 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 lysate
8. RSC Plunger

Figure 1. Maxwell® RSC Pathogen Total Nucleic Acid Cartridge. Preprocessed sample is added to well #1, and a plunger is added to well #8.

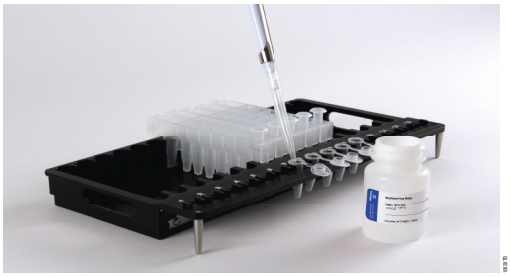


Figure 2. Setup and configuration of the deck tray(s). Elution Buffer is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

5. Maxwell® Instrument Run

For detailed information, refer to the Technical Manual specific to your Maxwell® Instrument. See Table 1.

1. Turn on the Maxwell® Instrument and Tablet PC. Log 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-check and home all moving parts.
2. Touch **Start** on the 'Home' screen.
3. On the 'Methods' screen, select a method using one of the two options below:
   a. Touch the RSC Pathogen TNA method.
   b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate method.
4. Verify that the RSC Pathogen TNA method has been selected, and touch the **Proceed** button. If requested by the software, enter any kit lot and expiration information that has been required by the Administrator.
5. On the 'Cartridge Setup' screen, confirm that the Maxwell® RSC Pathogen TNA method is displayed at the top of the screen. Touch the cartridge positions to select or deselect any positions to be used for this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.

   Note: When using the Maxwell® RSC 48 Instrument, press the **Front** or **Back** button to select or deselect cartridge positions for the appropriate 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(s): 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.

Note: If using a 48-position Maxwell® Instrument and the Vision System has been enabled, the deck trays will be scanned as the platform 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.

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

   Notes:

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

   b. 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, you should 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.

9. When the run is complete, the user interface will display a message that the method has ended.

End of Run

10. 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 have not been removed from the plunger bar, follow the instructions in the Technical Manual appropriate to your Maxwell® Instrument (see Table 1) to perform a **Clean Up** process to attempt to unload the plungers.

11. Remove the deck tray(s) from the instrument immediately following the run to prevent evaporation of the eluates. Remove elution tubes containing DNA, and cap the tubes.

    Note: Following the automated purification procedure, the deck tray(s) will be warm. To remove a deck tray from the instrument platform, hold onto the deck tray by its sides.

    Ensure samples are removed from the instrument before running a UV sanitation protocol to avoid damage to the nucleic acid.

12. Remove the cartridges and plungers from the Maxwell® deck tray(s). Discard as hazardous waste according to your institution's procedures. Do not reuse Maxwell® RSC Cartridges, RSC Plungers or Elution Tubes.

6. Storing Eluted Nucleic Acid

If samples are not processed immediately, store eluted pathogen DNA on ice or at 4°C for up to 24 hours. For longer term storage, freeze at –20°C or –70°C. RNA is less stable and preferably tested in downstream assays immediately after isolation. If testing soon after isolation, store pathogen RNA on ice or at 4°C. Alternatively, store eluted pathogen RNA at –70°C. Consult the instructions for downstream applications for specific sample storage and handling recommendations.

7. Creating a Ribonuclease-Free Environment

Ribonucleases are extremely difficult to inactivate. Take care to avoid introducing RNase activity into the samples of total nucleic acid that contain RNA 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 often to avoid ribonuclease contamination.
2. Whenever possible, sterile, disposable plasticware should be used for handling total nucleic acid that contains RNA. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
3. Treat non-sterile 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% v/v 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.

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

8. References

1. Clinical Laboratory Standards Institute (2020) CLSI MM13–Collection, transport, preparation and storage of specimens for molecular methods. Second Edition**.**
2. Baron, E.J. (2015) Specimen collection, transport, and processing: Bacteriology. In: *Manual of Clinical Microbiology, 11th Edition*, edited by Jorgensen, J.H. *et al*. 270–315. Washington, D.C., ASM Press.
3. Centers for Disease Control and Prevention. *Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing*. Accessed November 2, 2022: **www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html**
4. Centers for Disease Control and Prevention. *Stool Specimens - Molecular Diagnosis*. Accessed November 2, 2022: **www.cdc.gov/dpdx/diagnosticprocedures/stool/moleculardx.html**

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
Lower pathogen nucleic acid recovery than expected (e.g., for customer-provided internal controls)	The starting samples were compromised. Ensure that samples were collected, shipped and stored according to recommended guidelines.
	For RNA viral samples, ensure RNase-free conditions are used for sample preparation and assay setup, including RNase-free tubes and pipette tips.
	Processing step was not optimal. • Use only the Lysis Buffer provided with this kit. • Incomplete mixing may reduce lysis. Vortex sample with Lysis Solution as recommended. • Incomplete protease treatment to remove viral capsids and/or proteins from bacteria and parasites. Check the heat block or water bath temperature, and incubate for the full time recommended. • When working with difficult to lyse pathogens (Gram positive bacteria or parasites) in stool, the optional bead beating step can be used. • Make sure that 1-Thioglycerol has been added for BAL or sputum sample preprocessing. Omitting 1-Thioglycerol may negatively affect yields. • Adding more sample than recommended may reduce nucleic acid recovery. • Add an internal control to the sample after adding Lysis Buffer. The Lysis Buffer inactivates nucleases in the sample which may otherwise degrade the internal control.
	Check that a plunger was added to the cartridge.
	Post-purification storage issues. • Remove eluates, and store at the recommended temperature immediately after the Maxwell® Instrument run. • Do not subject eluates to multiple freeze-thaw cycles before downstream assays.
	Nucleic acid internal controls smaller than 100bp may not be efficiently purified using the system. The user is responsible for establishing performance of any internal control.

9. Troubleshooting (continued)

Symptoms	Causes and Comments
Poor amplification	Paramagnetic particle carryover may cause interference in amplification reactions. Remove particles in elution tube by centrifugation or magnetic separation.
	Wrong elution buffer was added. Use only the Elution Buffer supplied with the Maxwell® RSC Pathogen Total Nucleic Acid Kit.
	Inhibitor carryover from stool samples. Dilute viral RNA from stool samples 1:8 in nuclease-free water.
Cross-contamination	Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
	Avoid splashing when adding lysates to cartridges. Cartridges may be removed from the deck tray for sample addition to minimize contamination of adjacent cartridges.
Instrument unable to pick up plungers	Make sure you are using a Maxwell® RSC-specific chemistry kit; the plungers for the Maxwell® RSC reagent kits are specific to the supported Maxwell® Instruments for this kit.

10. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
RSC/CSC Plungers	50/pack	AS1331
Maxwell® RSC Plunger Pack	48/pack	AS1670
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® FSC Deck Tray	1 each	AS4016
Elution Tubes (0.5ml)	50/pack	AS6201
Elution Magnet, 16 Position	1 each	AS4017
Elution Magnet, 24 Position	1 each	AS4018
Microtubes, 1.5ml	1,000/pack	V1231
Clearing Columns	50 each	Z3871
RNase A Solution	1ml	A7973
	5ml	A7974
Proteinase K (PK) Solution	4ml	MC5005
Nuclease-Free Water	50ml	P1193

Maxwell® RSC Reagent Kits

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

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