

TECHNICAL MANUAL

Maxwell[®] RSC Genomic DNA Kit

Instructions for Use of Product
AS1880

Note: To use the Maxwell[®] RSC Genomic DNA Kit, you must have the “Genomic DNA” method loaded on the Maxwell[®] Instrument.

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

Maxwell[®] RSC Genomic DNA 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 Genomic DNA Kit^(a) is used with the Maxwell® Instruments specified in Table 1 to provide an easy method for efficient, automated sample preparation and purification of genomic DNA (gDNA) from a variety of human biological samples. The Maxwell® Instruments are designed for use with predisposed reagent cartridges and preprogrammed purification methods, maximizing simplicity and convenience. The Maxwell® method for the RSC Genomic DNA Kit can process from one to the maximum sample number in 40 minutes. The purified DNA can be used directly in a variety of downstream applications such as PCR-based assays.

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 Genomic DNA Kit purifies nucleic acid from samples using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of gDNA. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind nucleic acids to the paramagnetic particles in the first well of a prefilled cartridge. The samples are processed through a series of washes before the gDNA is eluted. This magnetic capture approach avoids common problems such as clogged tips or partial reagent transfers that result in suboptimal purification processing by other commonly used automated systems.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Genomic DNA Kit	48 preps	AS1880

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:

- 2 × 1ml Proteinase K (PK) Solution
- 1ml RNase A Solution
- 20ml Lysis Buffer
- 20ml Lytic Enhancer (LE2)
- 48 Maxwell® RSC Cartridges
- 1 Maxwell® RSC Plunger Pack (48 plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® RSC Genomic DNA Kit at +15°C to +30°C.



Safety Information: The Maxwell® RSC Cartridges contain ethanol and isopropanol. These substances should be considered flammable, harmful and irritants. Refer to the Safety Data Sheet (SDS) for detailed safety information. Adhere to institutional guidelines for the handling and disposal of all chemical waste used with this system.



The Maxwell® RSC Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and safety glasses) 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; seal edges may be sharp.

Additional Information: For additional safety information, see the Safety Data Sheet, available at: www.promega.com

3. Sample Preparation

Materials to Be Supplied by the User

- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for sample incubation are recommended (e.g., Microtubes, 1.5ml [Cat.# V1231]) Other tube types should be evaluated by the laboratory.
- dry heat block, water bath or thermal mixer set at 56°C
- deionized or Nuclease-Free Water (Cat.# P1193) for cell pellet (Section 3.B) and tissue samples (Section 3.D)
- 1X phosphate-buffered saline (PBS) for cell pellet samples prepared from urine (Section 3.B)
- **optional:** Clearing Columns (Cat.# Z3871) for buccal swab samples (Section 3.E)
- **optional:** rotating tube mixer

3.A. Preparing Lysates from Whole Blood and Buffy Coat Samples

The total genomic DNA yield from whole blood and buffy coat samples depends on the sample volume and number of white blood cells (WBC) per milliliter. For these sample types, a sample volume range of 50–300µl can be used. During development, whole blood and buffy coat prepared from whole blood with a range of 4×10^6 to 10×10^6 WBC/ml were tested and found to provide acceptable performance. Samples outside of this range may be compatible with the extraction chemistry but should be evaluated by the laboratory for performance and compatibility with downstream assays.

Use an elution volume of 50–200µl for whole blood samples. Because buffy coat samples generally yield a high amount of genomic DNA, we recommend eluting with 200µl to provide the most effective elution. Elution volumes of 50–200µl can be used with buffy coat samples but volumes less than 200µl may not provide optimal results.


Notes:

- a. This kit has been tested with human whole blood and buffy coat samples prepared from human whole blood collected in EDTA, sodium citrate or heparin tubes. Performance of this kit with other types of blood collection tubes should be evaluated by the user.
 - b. This kit has been tested with blood and buffy coat samples stored under the following conditions: Stored at 15–30°C for up to 72 hours, stored at 2–10°C for up to 7 days or stored at –65°C or lower prior to DNA purification. Other sample storage conditions may provide acceptable performance but should be evaluated by the laboratory. Completely thaw frozen samples before processing. All blood and buffy coat samples should be thoroughly mixed before use.
1. Mix all blood or buffy coat samples for at least 5 minutes at 15–30°C. This can be accomplished using a rotating tube mixer or by intermittent mixing with a vortex mixer.
 2. Prepare and label incubation tubes that will fit in a heat block set at 56°C.
 3. Add 30µl of Proteinase K (PK) Solution to each incubation tube.

4. Transfer the desired sample volume to each incubation tube. Change pipette tips between each sample transfer to prevent cross contamination.

Note: Transferring clotty, fatty or other solid material into the incubation tube can result in poor sample lysis. Only transfer liquid sample to the incubation tube.

5. Vortex each tube at maximum speed for 10 seconds.
6. Add 300µl of Lytic Enhancer (LE2) to each incubation tube. Change pipette tips each time the Lytic Enhancer (LE2) is dispensed to prevent cross contamination.

 Proceed to Step 7 without mixing or vortexing.

7. Add 300µl of Lysis Buffer to each incubation tube. Change pipette tips each time the Lysis Buffer is dispensed to prevent cross contamination.

8. Vortex each tube at maximum speed for 10 seconds.

Note: Confirm vortexing resulted in a homogeneous lysate.

9. Incubate each tube in the 56°C heat block for 20 minutes. During this incubation, prepare the Maxwell® RSC cartridges as described in Section 3.F.

10. Vortex each tube at maximum speed for 10 seconds.

11. Transfer each lysate sample from the incubation tube to well #1 (the largest well in the cartridge) of a separate cartridge and mix with the binding solution in well #1 by aspirating and dispensing 5–10 times to make a homogeneous mixture. Change pipette tips between each sample transfer to prevent sample cross contamination.


Note: Failure to create a homogeneous mixture of sample lysate and binding solution in well #1 of the cartridge may result in decreased yield and purity of the final eluate.

3.B. Preparing Lysates from Bone Marrow Aspirate Samples

The total genomic DNA yield from bone marrow aspirate samples depends on the total number of cells being processed. During development, bone marrow aspirate samples in the volume range of 50–300µl were tested and found to provide acceptable performance. Samples outside of this range may be compatible with the extraction chemistry but should be evaluated by the laboratory for performance and compatibility with downstream assays.

Because bone marrow aspirate samples generally yield a high amount of genomic DNA, we recommend eluting with 200µl to provide the most effective elution. Elution volumes of 50–200µl can be used with bone marrow samples but volumes less than 200µl may not provide optimal results.

Notes:

- a. This kit has been tested with human bone marrow aspirate samples collected in EDTA, sodium citrate or heparin tubes. Performance of this kit with other types of blood collection tubes should be evaluated by the user.
 - b. This kit has been tested with bone marrow aspirate samples stored frozen (stored at –65°C or lower) prior to DNA purification. Other sample storage conditions may provide acceptable performance but should be evaluated by the laboratory. Completely thaw frozen samples before processing. All bone marrow aspirate samples should be thoroughly mixed before use.
1. Mix all bone marrow aspirate samples for at least 30 minutes at 15–30°C using a rotating tube mixer or intermittently mixing with a vortex mixer.
 2. Prepare and label incubation tubes that will fit into a heat block set at 56°C.
 3. Add 30µl of Proteinase K (PK) Solution to each incubation tube.
 4. Transfer the desired sample volume to each incubation tube. Change pipette tips between each sample transfer to prevent cross contamination.
Note: Transferring clotty, fatty or other solid material into the incubation tube can result in poor sample lysis. Transfer only liquid sample to the incubation tube.
 5. Vortex each tube at maximum speed for 10 seconds.
 6. Add 300µl of Lytic Enhancer (LE2) to each incubation tube. Change pipette tips each time the Lytic Enhancer (LE2) is dispensed to prevent cross contamination.
 Proceed to Step 7 without mixing or vortexing.
 7. Add 300µl of Lysis Buffer to each incubation tube. Change pipette tips each time the Lysis Buffer is dispensed to prevent cross contamination.
 8. Vortex each tube at maximum speed for 10 seconds.
Note: Confirm vortexing resulted in a homogeneous lysate.
 9. Incubate each tube in the 56°C heat block for 20 minutes. During this incubation, prepare the Maxwell® RSC cartridges as described in Section 3.F.
 10. Vortex each tube at maximum speed for 10 seconds.

11. Transfer each lysate sample from the incubation tube to well #1 (the largest well in the cartridge) of a separate cartridge and mix with the binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture. Change pipette tips between each sample transfer to prevent sample cross contamination.

Note: Failure to create a homogeneous mixture of sample lysate and binding solution in well #1 of the cartridge may result in decreased yield and purity of the final eluate.

3.C. Preparing Lysates from Cell Pellet Samples

Cell pellets can be generated from a number of sample types including biological fluids (e.g., urine or amniotic fluid), purified cells (e.g., peripheral blood mononuclear cells) or cultured cells. Centrifugation of the sample is used to generate a cell pellet and that pellet is resuspended in 300µl of nuclease-free water. The total genomic DNA yield from cell pellet samples depends on the number of cells present in the sample. During development, cell pellets from up to 5×10^6 cells were tested (see Table 2) and found to provide acceptable performance. Samples outside of this range may be compatible with the extraction chemistry but should be evaluated by the laboratory for performance and compatibility with downstream assays.

Table 2. Cell Pellet Sample Types Evaluated.

Sample Type	Sample Range Tested	Suggested Elution Volume
Urine	15–50ml	50µl
Amniotic fluid	1–5ml	50µl
Peripheral blood mononuclear cells (PBMC)	5×10^4 – 5×10^6 cells	50–200µl
Cultured cells	5×10^2 – 5×10^6 cells	50–200µl


For cell pellet samples, use an elution volume range of 50–200µl. When processing samples that generate low cell numbers in the pellet, we recommend a 50µl elution volume. For samples with more cells, a larger elution volume can result in higher genomic DNA yields. Laboratories should confirm that the elution volume for a given sample type provides sufficient purity and concentration for their downstream assay.

Notes:

- a. This kit has been tested with cell pellet samples processed immediately after generating a cell pellet and stored frozen (stored at -65°C or lower) prior to DNA purification. Other sample storage conditions may provide acceptable performance but should be evaluated by the laboratory. Completely thaw frozen samples before processing.
- b. If sample freezing is desired, samples should be frozen after generating the cell pellet. Collecting a cell pellet from a sample that has been frozen and thawed can result in loss of performance.

3.C. Preparing Lysates from Cell Pellet Samples (continued)

1. Centrifuge the desired sample volume at a minimum of $2,000 \times g$ for 20 minutes to generate a cell pellet.
 - a. For urine samples, wash the cell pellet by resuspending in 750 μ l of 1X PBS.
 - b. Centrifuge the PBS-suspended sample at a minimum of $2,000 \times g$ for 20 minutes to generate a cell pellet.
2. Decant or aspirate the liquid from the pelleted cells. Resuspend the pellet in 300 μ l of Nuclease-Free Water (Cat.# P1193 or equivalent).
3. Prepare and label incubation tubes that will fit into a heat block set at 56°C.
4. Add 30 μ l of Proteinase K (PK) Solution to each incubation tube.
5. Transfer the desired sample volume to each incubation tube. Change pipette tips between each sample transfer to prevent cross contamination.

Note: Transferring clotty, fatty or other solid material into the incubation tube can result in poor sample lysis. Only transfer liquid sample to the incubation tube.
6. Vortex each tube at maximum speed for 10 seconds.
7. Add 300 μ l of Lytic Enhancer (LE2) to each incubation tube. Change pipette tips each time the Lytic Enhancer (LE2) is dispensed to prevent cross contamination.
-  Proceed to Step 8 without mixing or vortexing.
8. Add 300 μ l of Lysis Buffer to each incubation tube. Change pipette tips each time the Lysis Buffer is dispensed to prevent cross contamination.
9. Cap and vortex each tube at maximum speed for 10 seconds.

Note: Confirm vortexing resulted in a homogeneous lysate.
10. Incubate each tube in the 56°C heat block for 20 minutes. During this incubation, prepare the Maxwell[®] RSC cartridges as described in Section 3.F.
11. Vortex each tube at maximum speed for 10 seconds.
12. Transfer each lysate sample from the incubation tube to well #1 (the largest well in the cartridge) of a separate cartridge and mix with the binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture. Change pipette tips between each sample transfer to prevent sample cross contamination.

Note: Failure to create a homogeneous mixture of sample lysate and binding solution in well #1 of the cartridge may result in decreased yield and purity of the final eluate.

3.D. Preparing Lysates from Tissue Samples

The total genomic DNA yield from tissue samples depends on the mass and type of tissue processed. For tissue samples, a sample range of 5–50mg can be used. During development, heart, pancreas, brain and breast tissue samples were evaluated as exemplars and found to provide acceptable performance. A wider range of tissue types may be compatible with the extraction chemistry but should be evaluated by the laboratory for performance and compatibility with downstream assays.

Use an elution volume of 50–200µl for tissue samples. The volume of elution buffer to use will depend on the mass and type of tissue being processed. Laboratories should evaluate elution volumes that provide acceptable performance in their downstream assays for the tissue mass and types being processed.

Note: This kit has been tested with tissue samples stored frozen (stored at –65°C or lower) prior to DNA purification. Other sample storage conditions may provide acceptable performance but should be evaluated by the laboratory. Completely thaw frozen samples before processing.

1. To lyse tissue samples, set the temperature of a dry heat block, water bath or thermal mixer to 56°C. Prepare and label incubation tubes that will fit in the desired heating option.
2. Transfer 5–50mg of tissue to each tube. Cutting tissue into smaller fragments may decrease lysis time. Centrifuge the tube at top speed for 15 seconds to collect the tissue pieces at the bottom of the tube.
3. Add 300µl of Nuclease-Free Water (Cat.# P1193 or equivalent) to each incubation tube.
4. Add 30µl of Proteinase K (PK) Solution to each incubation tube. Change pipette tips each time the Proteinase K (PK) Solution is dispensed to prevent cross contamination.
5. Vortex each tube at maximum speed for 10 seconds.
6. Add 300µl of Lytic Enhancer (LE2) to each incubation tube. Change pipette tips each time Lytic Enhancer (LE2) is dispensed to prevent cross contamination.
7. Vortex each tube at maximum speed for 10 seconds.
8. Incubate each tube at 56°C using one of the following options:
 - a. With a thermal mixer, use a high shake speed (e.g., 1,500rpm) for up to 2 hours.
 - b. With a dry heat block or water bath heater, use without shaking for at least 16 hours.
9. Vortex each tube at maximum speed for 10 seconds.
10. Centrifuge each tube at maximum speed in a microcentrifuge for 5 minutes to pellet any undigested material.
11. Transfer all of the supernatant from each incubation tube into a new tube. Avoid transferring any pelleted material. If a distinct fatty layer appears on top of the sample after centrifugation, do not transfer that layer to the new tube.
12. Add 300µl of Lysis Buffer to each new tube. Change pipette tips each time the Lysis Buffer is dispensed to prevent cross contamination.
13. Vortex each tube at maximum speed for 10 seconds.

3.D. Preparing Lysates from Tissue Samples (continued)

14. Prepare cartridges as described in Section 3.F.
15. Transfer the tissue lysate sample from each tube to well #1 (the largest well in the cartridge) of a separate cartridge and mix with the binding solution in well #1 by aspirating and dispensing at least 10 times after transfer to make a homogeneous mixture. Change pipette tips between each sample transfer to prevent sample cross contamination.

Notes:

- a. Transferring the tissue pellet or fatty layer from the incubation tube into the new tube may result in poor yield or purities.
- b. Failure to create a homogeneous mixture of sample lysate and binding solution in well #1 of the cartridge can result in decreased yield and purity in the final eluate.

3.E. Preparing Lysates from Buccal Swab Samples

The total genomic DNA yield from buccal swab samples depends on how well buccal cells are transferred to the swab. During development, 1 and 2 buccal swabs were tested and provided acceptable performance. An elution volume range of 50–200µl can be used for buccal swab samples. Laboratories should choose an elution volume for buccal swab samples that provides sufficient purity and concentration for their downstream assay.

Note: This kit has been tested with dry buccal swab samples stored at 15–30°C prior to DNA purification. Other sample storage conditions may provide acceptable performance but should be evaluated by the laboratory.

1. Prepare and label 1.5–2.0ml incubation tubes that will fit in a heat block set at 56°C.
2. **Optional:** Place a clearing column (Cat.# Z3871) into each incubation tube.
3. Place 1–2 buccal swab head(s) in each incubation tube or clearing column in each incubation tube. Remove the stick from the buccal swab head(s) by cutting or breaking the swab stick off above the swab head so that the cap can be closed on the tube or clearing column containing the swab head.
4. In a separate tube, combine 300µl of Lytic Enhancer (LE2) with 30µl of Proteinase K (PK) Solution for each sample plus one extra sample. See the table below. For example, to process 16 samples, create a master mix for 17 reactions by combining $300\mu\text{l} \times 17 = 5,100\mu\text{l}$ Lytic Enhancer (LE2) and $30\mu\text{l} \times 17 = 510\mu\text{l}$ Proteinase K.

Reagent	Amount Per Reaction	Reactions (Sample Number + 1)	Total
Lytic Enhancer (LE2)	300µl	n + 1	$300 \times (n + 1)\mu\text{l}$
Proteinase K (PK) Solution	30µl	n + 1	$30 \times (n + 1)\mu\text{l}$

5. Mix the Lytic Enhancer (LE2)/Proteinase K (PK) Solution by inverting the tube at least 10 times.
6. Add 330µl of Lytic Enhancer (LE2)/Proteinase K (PK) Solution to each sample, and close tube. Change pipette tips each time Lytic Enhancer (LE2)/Proteinase K (PK) Solution is dispensed to prevent cross contamination.
7. Incubate each tube at 56°C for 20 minutes. During this incubation, prepare cartridges as described in Section 3.F.
8. Use one of the following options to remove the swab head(s) from the tube:

- a. If using a Clearing Column, place the tube in a microcentrifuge and centrifuge at maximum speed for 2 minutes. Remove the tube from the microcentrifuge. Open the tube; remove and discard the clearing column containing the swab head(s).
 - b. If not using a Clearing Column, use tweezers to remove the swab head(s) from the tube, carefully squeezing the remaining lysate from the swab head(s). Discard the swab head(s). Clean the tweezers and change gloves between each swab head removal to prevent cross contamination.
9. Add 300µl of Lysis Buffer to well #1 of each cartridge to be used (well #1 is the largest well in the cartridge).
 10. Transfer each swab lysate sample from the incubation tube to well #1 of a separate cartridge and mix with the Lysis Buffer and binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture. Change pipette tips between each sample transfer to prevent sample cross contamination.
Note: Failure to create a homogeneous mixture of sample lysate, Lysis Buffer and binding solution in well #1 can result in decreased yield and purity in the final eluate.

3.F. Preparing the Maxwell® RSC Genomic DNA Cartridges

1. Change gloves before handling cartridges, RSC Plungers and Elution Tubes (0.5ml). Cartridges are set up in the deck tray(s) outside of the instrument before transferring the deck tray(s) containing the cartridges and samples to the instrument for purification. Place each cartridge in the deck tray(s) with well #1 (the largest well in the cartridge) farthest away from the elution tubes (Figure 2). Press down on the cartridge to snap it into position. Ensure both cartridge ends are fully seated in the deck tray. Carefully peel back the seal so that the entire seal is removed from the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed from the cartridge.



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

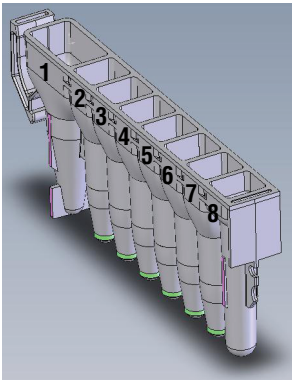
2. Add 15µl of RNase A Solution into well #3 of the Maxwell® RSC Cartridge.
3. Place one plunger into well #8 of each cartridge.
4. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s).
Note: Use only the elution tubes provided in the Maxwell® RSC Genomic DNA Kit. Other elution tubes may be incompatible with the Maxwell® RSC Instruments and affect DNA purification performance.
5. Add 50–200µl of Elution Buffer to the bottom of each elution tube.
Note: Only use the Elution Buffer provided in the Maxwell® RSC Genomic DNA Kit. Use of other elution buffers may affect DNA purification performance.
6. Proceed to Section 4, Maxwell® Instrument Run.

3.F. Preparing the Maxwell[®] RSC Genomic DNA Cartridge (continued)

Maxwell[®] RSC Genomic DNA Cartridge Preparation Notes:



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 any instrument parts.



User Adds to Wells

1. Lysed sample
3. 15µl of RNase A Solution
8. RSC Plunger

Figure 1. Maxwell[®] RSC Cartridge. Lysed sample is added to well #1, 15µl of RNase A Solution is added to well #3, 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 indicated. Deck tray shown is from the Maxwell[®] RSC Instrument (Cat.# AS4500).

4. 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 Genomic DNA 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 Genomic DNA 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 Genomic DNA 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.

4. Maxwell® Instrument Run (continued)

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.



5. 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

Lower than expected DNA concentration

Causes and Comments

Samples that have undergone multiple freeze-thaw cycles may have degraded DNA. Sample guidelines for collection and storage are listed for each specific sample type.

Sample contained a low amount of genomic DNA. The genomic DNA yield depends on the amount of sample being processed and the DNA content of that sample.

Proteinase K Solution was not added, an incomplete volume of Proteinase K Solution was added, or the Proteinase K was not effectively mixed with the sample. Lysis and yield are dependent upon complete extraction with Proteinase K.

Input sample was not mixed before processing. Be sure to mix samples before processing.

Elution volume used for extraction was too large for the sample being processed. To increase eluted DNA concentration, reduce the initial elution buffer volume.

Too much sample or sample containing an excessive amount of genomic DNA was processed. Excessive sample or genomic DNA may cause extraction chemistry failure, resulting in an eluate concentration that does not correlate with the amount of sample being processed.

Lysate was not mixed with the binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture. Failure to create a homogeneous mixture of sample lysate and binding solution in well #1 can result in decreased yield and purity in the final eluate.

Samples were not mixed appropriately or at the correct steps during processing. Failure to adequately mix reagents and samples together in the incubation tube can affect performance.

Lysis Buffer and Lytic Enhancer (LE2) were used interchangeably, at the incorrect step or at the incorrect volume. Reprocess samples, correctly using Lysis Buffer and Lytic Enhancer (LE2) as instructed.

5. Troubleshooting (continued)

Symptoms

Causes and Comments

Lower than expected purity

Proteinase K Solution was not added, an incomplete volume of Proteinase K Solution was added, or the Proteinase K was not effectively mixed with the sample. Lysis and yield are dependent upon complete extraction with Proteinase K.

Lysate was not mixed with the binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture. Failure to create a homogeneous mixture of sample lysate and binding solution in well #1 can result in decreased yield and purity in the final eluate.

Samples that have undergone multiple freeze-thaw cycles may have degraded DNA. Use samples that have been collected and stored under the guidelines listed under each specific sample type.

For whole blood, buffy coat, and bone marrow samples, transferring clotty or fatty material into the incubation tube can result in poor lysis of the sample. Transfer only liquid samples for purification.

Lysis Buffer and Lytic Enhancer (LE2) were used interchangeably, at the incorrect step or at the incorrect volume. Reprocess samples, correctly using Lysis Buffer and Lytic Enhancer (LE2) as instructed.

Some tissue types can produce lower than expected purity values. If higher purity values are desired, reduce the input amount of tissue processed.

Samples were not mixed appropriately or at the correct steps during processing. Failure to adequately mix reagents and samples together in the incubation tube can affect performance.

Transferring solid material into well #1 of the cartridge can result in copurification of solid material and contaminants. Remove solid material before transferring lysed sample into the cartridge.

RNA contamination

RNase A Solution was not added to well #3 of the cartridge or an incomplete volume of RNase A Solution was added. Reprocess sample with RNase A Solution or treat extracted gDNA sample with RNase A.

Resin carryover

Samples were not mixed appropriately or during the correct steps during processing. Failure to adequately mix reagents and samples together in the incubation tube or well #1 can affect resin carryover in the cartridge and elution tube.

Symptoms

Resin carryover (continued)

Causes and Comments

Too much sample or sample containing an excessive amount of genomic DNA was processed. Excessive sample may cause excess resin carryover in the cartridge and elution tube.

Some resin carryover is normal and does not affect downstream performance. If necessary, use an Elution Magnet ([Cat.# AS4017, Cat.# AS4018 or both]; available separately) to transfer the eluate into a new tube. See Section 6, Related Products.

6. Related Products
Instruments 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

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



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