

TECHNICAL MANUAL

Maxwell[®] RSC Whole Blood DNA Kit

Instructions for Use of Products
AS1520 and ASB1520

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

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

Maxwell[®] RSC Whole Blood DNA Kit

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

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1. Description

The Maxwell® RSC Whole Blood DNA Kit^(a) is used with the Maxwell® and Maxprep® Instruments specified below to provide a simple method for efficient, automated sample preparation and purification of genomic DNA (gDNA) from human whole blood samples. Maxwell® Instruments are supplied with preprogrammed purification procedures and are designed for use with predisposed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. Maxwell® methods for the RSC Whole Blood 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, including PCR.

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, AS9105 AS9200, AS9201, AS9205	TM509

The Maxwell® RSC Whole Blood DNA Kit purifies 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 gDNA to the paramagnetic particle in the first well of a prefilled cartridge. The samples are processed through a series of washes before the gDNA is eluted.

Prior to extraction, samples can be preprocessed manually or using the Maxprep® Liquid Handler. The Maxprep® Liquid Handler will transfer samples from primary blood tubes to Maxwell® RSC Whole Blood DNA cartridges, transfer plungers to Maxwell® RSC Whole Blood DNA 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 Whole Blood DNA Kit	48 preps	AS1520

For Research Use Only. Not for use in diagnostic procedures. Contains sufficient reagents for 48 automated isolations from 50µl to 500µl of whole blood samples. Cartridges are for single use only. Includes:

- 48 Maxwell® RSC Cartridge (RSCH)
- 1 Maxwell® RSC Plunger Pack (48 plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

PRODUCT	SIZE	CAT.#
Maxwell® RSC Whole Blood DNA Multi-Pack Kit	144 preps	ASB1520

For Research Use Only. Not for use in diagnostic procedures. Contains sufficient reagents for 144 automated isolations. Cartridges are single-use only. **Note:** ASB1520 is not recommended for use with the Maxprep® Liquid Handler. Includes:

- 144 Maxwell® RSC Cartridge (RSCH)
- 3 × 50/pkg Maxwell® CSC/RSC Plungers
- 3 × 50 Elution Tubes (0.5ml)
- 3 × 20ml Elution Buffer

Storage Conditions: Store the Maxwell® RSC Whole Blood DNA Kit at ambient temperature (+15°C to +30°C).

Safety Information: The Maxwell® RSC Cartridges contain ethanol, isopropanol, guanidine hydrochloride and guanidine thiocyanate. Ethanol and isopropanol should be considered flammable, harmful and irritants. Guanidine thiocyanate and guanidine hydrochloride should be considered toxic, harmful and irritants. Refer to the Safety Datasheet (SDS) for detailed safety information.



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. Bleach reacts with guanidine thiocyanate and should not be added to any sample waste from these cartridges.

Available Separately For Preprocessing with the Maxprep® Liquid Handler

PRODUCT	SIZE	CAT.#
Maxprep® 1000µl Conductive Disposable Tips, Filtered	40/box	AS9303
Maxprep® 300µl Conductive Disposable Tips, Filtered	60/box	AS9302
Maxprep® Reagent Reservoir, 50ml	28/pack	AS9304
Maxprep® Plunger Holder	1 each	AS9408
Maxwell® RSC Plunger Pack	1 each	AS1670



3. Sample Preparation

Materials to Be Supplied by the User

- rotating tube mixer for liquid blood samples

The total yield of genomic DNA from whole blood samples depends on the sample volume and number of white blood cells/ml. Each Maxwell® RSC Cartridge supplied in the Maxwell® RSC Whole Blood DNA Kit is designed to purify genomic DNA from 50µl to 500µl of whole blood, assuming an average number of white blood cells in the range of 4×10^6 to 1.1×10^7 cells/ml whole blood (values for a normal healthy adult; 1).

Whole blood samples collected in EDTA, ACD or heparin tubes can be used. These samples may be either fresh or frozen. Frozen samples should be thawed before processing. We highly recommend mixing all blood samples for at least 5 minutes at room temperature before use.

4. Manual Preprocessing

4.A. Maxwell® RSC Whole Blood DNA Cartridge Preparation

Materials to Be Supplied by the User

- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
1. 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). Place each cartridge 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. Transfer 50–500µl of each blood sample from the starting blood tube to well #1 of each cartridge (well #1 is the largest well).
 3. Tip-mix the blood sample in well #1 to ensure all blood has been transferred. Change pipette tips between samples.
 4. Place one plunger into well #8 of each cartridge.
 5. Place an empty elution tube into the elution tube position for each cartridge in the deck tray. Add 60µl of Elution Buffer to the bottom of each elution tube.
 6. Proceed to Section 6, Maxwell® Instrument Setup and 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 bacteriocidal spray or wipe and then water. Do not use bleach on any instrument parts.
- b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.
- c. If a larger volume of eluate is desired, the purified nucleic acid can be eluted in up to 150µl of Elution Buffer. Elution with 60µl of Elution Buffer will result in the greatest final **concentration** of purified nucleic acid, while elution with a higher volume will result in higher **yield** of purified nucleic acid (Figure 1).

4. The starting volume of Elution Buffer will not result in the same elution volume after running the method. Typically, the resulting elution volume will be approximately 15–25 μ l less than the starting volume.
5. Resin carryover into the Elution Tube is typical. Using a larger starting volume of Elution Buffer will reduce carryover.

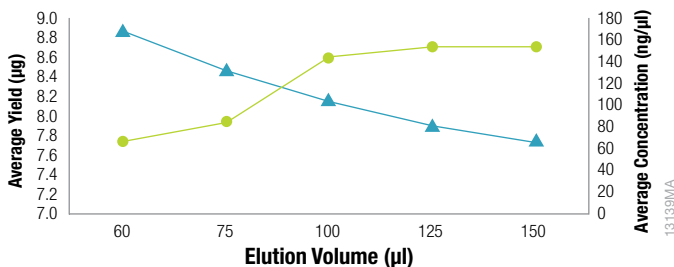
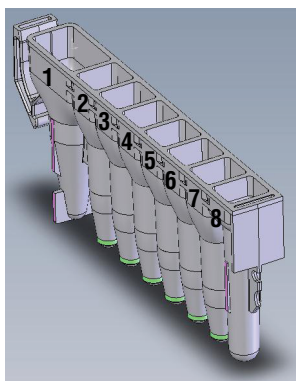


Figure 1. Effect of elution volume on yield and concentration. Average yield versus elution volume (circles). Average concentration versus elution volume (triangles).



User Adds to Wells

1. Whole blood sample (50–500 μ l)
8. RSC Plunger

Figure 2. Maxwell® RSC Cartridge.



Figure 3. 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. Maxprep® Preprocessing

5.A. Maxprep® Cartridge Preparation

1. Mix all blood samples for at least 5 minutes at room temperature.
2. 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.
3. Touch **Start** to access the 'Methods' screen.
4. On the 'Methods' screen, select a method using one of the two options below:
 - a. Touch the Whole Blood DNA preprocessing method or laboratory-specific variant of the Whole Blood DNA 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 Whole Blood DNA preprocessing method, if desired.
5. 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.
6. Enter any method-specific variables (Sample Number, Sample Volume, Elution Volume).

Notes:

- a. If a larger volume of eluate is desired, the purified nucleic acid can be eluted in up to 150µl of Elution Buffer. Elution with 60µl of Elution Buffer will result in the greatest final concentration of purified nucleic acid, while elution with a higher volume will result in higher yield of purified nucleic acid (Figure 1).
- b. The starting volume of Elution Buffer will not result in the same elution volume after running the method. Typically, the resulting elution volume will be approximately 15–25µl less than the starting volume.
- c. Resin carryover into the Elution Tube is typical. Use a larger starting volume of Elution Buffer to reduce carryover.

7. 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:

- a. 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.
 - b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.
8. 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:

Labware Type

- 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 (depending on sample number)
 - Maxprep® Reagent Reservoir, 50ml with Elution Buffer
 - Tube racks with sample tubes. All tubes within a carrier must be of the same type.
 - Maxprep® 1000µl Conductive Disposable Tips, Filtered (2; one may be partially full)
 - Maxprep® 300µl Conductive Disposable Tips, Filtered (racks may be partial or full)
9. Close the instrument door, and touch the **Next** button to start the automated preprocessing of samples.

5.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. Plungers are transferred to each of the cartridges in the Maxwell® deck tray(s). The specified volume of Elution Buffer is transferred to the elution tubes for each position in the Maxwell® deck tray(s).
2. The system transfers the specified volume of whole blood from each sample tube to its corresponding Maxwell® RSC cartridge.
3. 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 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.

6. Maxwell® Instrument Setup and Run

For detailed information, refer to the Operating 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 on the Tablet PC. The instrument will power up, proceed through a self-check 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 database, 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 **Whole Blood DNA** method.
4. If applicable to your Maxwell® Instrument, verify that the Whole Blood DNA method is selected, and press the **Proceed** button. If requested by the software, scan or enter any kit lot and expiration information that has been required by the Administrator.
5. On the 'Cartridge Setup' screen, touch the cartridge positions to select or deselect the positions that will be used for this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.
Note: When using 48-position Maxwell® Instruments, press the **Front** and **Back** buttons to select or 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(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: When using a 48-position Maxwell® Instrument, if the Vision System has been enabled, the deck trays 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.

8. 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:

- 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 are not 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. Remove elution tubes containing DNA, and cap the tubes. After the run has been completed, 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.

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



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

7. Reference

1. Henry, J.B. (2001) *Clinical Diagnosis and Management by Laboratory Methods*, 20th ed., W.B. Saunders Company, 509.

8. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com. Email: techserv@promega.com

Symptoms

Causes and Comments

Lower than expected A_{260}
(lower than expected yield)

Blood that has undergone multiple freeze-thaw cycles may have degraded DNA. Use fresh samples whenever possible, or avoid multiple freeze-thaw cycles.

Whole blood sample contained low white blood cell count. The yield of genomic DNA from blood samples depends on the number of white blood cells present in the sample.

Whole blood sample was not mixed before processing. Be sure to mix whole blood samples before processing to ensure that the white blood cells are in suspension.

The incorrect method was chosen for extraction on the Maxwell® Instrument. Ensure that the correct method is chosen.

Lower than expected purity ratios
(low A_{260}/A_{280} or A_{260}/A_{230} ratios)

Blood has been stored unfrozen for an extended period of time or has undergone multiple freeze-thaw cycles. Avoid these storage conditions.

The Maxwell® Instrument was set for the wrong method. Ensure that the Whole Blood DNA method is chosen.

RNA contamination in DNA eluates

In some cases, total RNA can be copurified with the genomic DNA. To remove copurified RNA, an RNase treatment can be performed. Add 5µl of RNase A (Cat.# A7973) per milliliter of Elution Buffer.

Instrument unable to pick up plungers

Make sure you are using an RSC-specific kit; the plungers for the Maxwell® RSC reagent kits are specific to the supported Maxwell® Instruments for this kit (see Section 1).

9. 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® FSC Deck Tray	1 each	AS4016
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
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 w/ UV light	1 each	AS9105
Maxprep® Liquid Handler, RSC 48 Carriers w/ UV light	1 each	AS9205
2.0ml Deep Well Plates (Non-Sterile)	60/pack	AS9309
Nunc™ 2.0ml Deep Well Plates	60/pack	AS9307
Maxprep® 1000µl Conductive Disposable Tips, Filtered	40/box	AS9303
Maxprep® 300µl Conductive Disposable Tips, Filtered	60/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
RNase A Solution, 4mg/ml	1ml	A7973
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



10. Summary of Changes

The following changes were made to the 1/25 revision of this document:

1. Added Cat.# AS9105 and AS9205 to Table 1 and Section 9.
2. Updated Maxprep to a registered trademark.
3. Edited Section 6 for consistency with other Maxwell® RSC manuals.
4. Updated the document font.
5. Removed the disclaimer.

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