

TECHNICAL MANUAL

Maxwell[®] 16 DNA Purification Kits

Instructions for Use of Products AS1010 and AS1030

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



Maxwell® 16 DNA Purification Kits

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® 16 DNA Purification Kits are intended for general laboratory use in combination with a Maxwell® 16 Instrument to perform automated isoloation of genomic DNA (gDNA) from whole blood, buffy coat or tissue samples. Maxwell® 16 Instruments are supplied with preprogrammed purification procedures and is designed for use with the predispensed reagent cartridges, maximizing simplicity and convenience. The instrument can process up to 16 samples in 30–40 minutes. The purified gDNA can be used directly in a variety of downstream applications including PCR and agarose gel electrophoresis.



1. Description (continued)

Maxwell® 16 Instruments purify samples using paramagnetic particles (PMPs), which provide a mobile solid phase that optimizes capture, washing and elution of the target material. Maxwell® 16 Instruments are magnetic particle handlers that efficiently preprocess liquid and solid samples, transport the PMPs through purification reagents in the prefilled cartridges (Figure 1) and mix during processing. The magnetic particle-based methodology 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

PROD	DUCT		SIZE	CAT.#
Maxwell® 16 Blood DNA Purification Kit			48 preps	AS1010
For L	aborator	/ Use. Sufficient for 48 automated isolations from whole blood or b	uffy coat samples. Includes:	:
•	48	Maxwell [®] 16 Blood DNA Cartridges		
•	50	Purification Plungers		
•	50	Elution Tubes		
•	20ml	Elution Buffer		
PRODUCT		SIZE	CAT.#	
Maxwell® 16 Tissue DNA Purification Kit 48 preps			AS1030	
For L	aborator	/ Use. Sufficient for 48 automated isolations from up to 50mg tissu	ie samples. Includes:	

- 48 Maxwell[®] 16 Tissue DNA Cartridges
- 50 Purification Plungers
- 50 Elution Tubes
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® 16 DNA Purification Kits at +15°C to +30°C.

Safety Information: The reagent cartridges contain ethanol, isopropanol and guanidine thiocyanate. These substances should be considered flammable, harmful and irritants.

The Maxwell[®] 16 reagent 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.



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The Maxwell[®] 16 reagent cartridges contain potentially hazardous chemicals. Wear gloves or other protective means when handling the reagent cartridges. Follow your institutional guidelines for disposal.



3. Maxwell[®] 16 Blood DNA Purification Kit (for use with Cat.# AS1010)

Materials to Be Supplied by the User

- · pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- storage tubes for purified DNA samples

3.A. Product Use Limitations

Intended Use: The Maxwell® 16 Blood DNA Purification Kit (Cat.# AS1010) is used in combination with Maxwell® 16 Instruments to perform automated isolation of genomic DNA from human whole blood or buffy coat samples. Samples collected in blood collection tubes treated with EDTA, heparin or citrate can be used. The nucleic acid isolation methodology used by the Maxwell® 16 Blood DNA Purification Kit produces DNA suitable for direct downstream analysis by standard amplification methods. These methods include a variety of polymerase chain reaction (PCR) tests for human in vitro diagnostic purposes. The Maxwell® 16 Blood DNA Purification Kit is not intended for use as part of a specific in vitro diagnostic test.

Product Use Limitations: The Maxwell[®] 16 Blood DNA Purification Kit (Cat.# AS1010) is not intended for use with tissue samples or samples from body fluids other than blood. It is not intended for purification of RNA. The Maxwell[®] 16 Blood DNA Purification Kit has been designed to isolate DNA from 50–400µl whole blood samples or 250µl buffy coat samples, obtained from healthy individuals with a white blood cell count ranging from 4.2 × 10⁶ to 1.2 × 10⁷ cells/ml. It is not intended for use with samples that have white blood cell counts outside this range.

The user is responsible for establishing performance characteristics necessary for downstream diagnostic applications. Include appropriate controls in any downstream diagnostic applications using DNA purified using the Maxwell® 16 Blood DNA Purification Kit.



3.B. Whole Blood Sample Processing Capacity and Yield

The total yield of genomic DNA from whole blood samples depends on the sample volume and number of white blood cells/ml. Each cartridge supplied in the Maxwell[®] 16 Blood DNA Purification Kit is designed to purify genomic DNA from up to 400µl of whole blood, assuming an average number of white blood cells in the range of 4.2×10^6 to 1.2×10^7 /ml whole blood (values for a normal healthy adult). Exceeding the recommended volume or using a sample with a white blood cell count outside of this range may adversely affect yield and quality of the purified genomic DNA.

Notes:

- a. Whole blood samples collected in EDTA, ACD or heparin can be used. These samples may be either fresh or frozen. Frozen samples should be thawed and mixed before processing.
- b. Fresh blood samples should be stored at 4°C and processed within 7 days of collection.
- c. After elution of concentrated gDNA samples, any residual MagneSil® particles can be removed by performing a second clearing using the Magnetic Elution Rack or by centrifugation of the eluted material followed by transfer of the supernatant to a fresh tube.

Table 1. Whole Blood Sample Volume and Preprocessing Requirements.

Sample Type	Kit	Volume	Preprocessing Requirements
Human whole blood Maxwell® 16 Blood DNA Purification Kit (Cat.# AS1010		50-400µl	None



3.C. Buffy Coat Sample Processing Capacity and Yield

Centrifugation of a whole blood sample at $2,000 \times g$ for 20 minutes results in separation of the material into three layers: a bottom layer containing mainly red blood cells, a top plasma layer and a thin white layer at the interface that is enriched for white blood cells. A 1ml pipette can be used to carefully collect the enriched white cells (buffy coat) from the interface. Typically this leads to a tenfold concentration of the white cells from a blood sample, depending on user technique and on how well the white cells pack. Characteristics such as sample age and storage, clarity of the plasma layer and white blood cell count can affect recovery of the buffy coat fraction and the resultant DNA yield.

Table 2. Buffy Coat Sample Volume and Preprocessing Requirements.

Sample Type Kit		Volume		Preprocessing Requirements	
Human buffy coat	Maxwell [®] 16 Blood DNA Purification Kit (Cat.# AS1010)	250µl concentrated from 2.5ml of whole blood	1. 2. 3.	Spin Vacutainer® tube for 20 minutes at 2,000 × g. Harvest white cells using a 1ml pipette. Add sample to well #1.	

A volume of 250µl of buffy coat (obtained from 2.5ml of whole blood) can be processed using the Maxwell® 16 Blood DNA Cartridge and the buffy coat method supplied on the Maxwell 16 Instrument.

Notes:



Elution volume is important.

Place 300µl of elution buffer into the Maxwell[®] 16 Elution Tube when processing 250µl of buffy coat sample. Some elution buffer will be lost during the run due to evaporation and absorption onto the MagneSil[®] particles during elution.

- b. After elution of concentrated genomic DNA, any residual MagneSil[®] particles can be removed by performing a second clearing using the Magnetic Elution Rack or by centrifuging the eluted material and removing the supernatant to a fresh tube.
- c. The concentration of the purified DNA should be measured by absorbance at 260nm. DNA purity should be confirmed by agarose gel electrophoresis and by measuring the A_{260}/A_{280} ratio, which is typically >1.7.
- Appropriate controls must be included in downstream diagnostic applications using DNA purified with the Maxwell[®] 16 system.



4. Maxwell® 16 Tissue DNA Purification Kit (for use with Cat.# AS1030)

4.A. Product Use Limitations

Intended Use: The Maxwell[®] 16 Tissue DNA Purification Kit is intended for use with the Maxwell[®] 16 Instruments for automated purification of genomic DNA from fresh or thawed tissue samples. DNA can be purified from tissue samples of up to 50mg. The purified DNA is suitable for use in direct downstream analysis by standard amplification methods. The Maxwell[®] 16 Tissue DNA Purification Kit is not intended for use as part of a specific in vitro diagnostic test.

Product Use Limitations: The Maxwell[®] 16 Tissue DNA Purification Kit is not intended for use with sample types other than tissue or for tissue samples greater than 50mg in size. It is not intended for the purification of RNA or non-genomic DNA.

The user is responsible for establishing performance characteristics necessary for the user's downstream applications. Include appropriate controls in any downstream applications using DNA purified using Maxwell® 16 products.

4.B. Tissue Sample Volume and Preprocessing Requirements

The total yield of genomic DNA from tissue samples depends on the sample size (weight) and tissue type. It is normal for different tissue types of the same mass to give different genomic DNA yields. The Maxwell® 16 Tissue DNA Purification Kit is designed to purify genomic DNA from up to 50mg of tissue. Exceeding this recommended sample size may adversely affect yield and quality of the purified genomic DNA.

Protocol

Place fresh or thawed tissue (up to 50mg) into well #1 of predispensed cartridge.

Note: Mouse-tail clippings longer than 0.5cm should be clipped in half to obtain maximal yield.

Optional RNase Treatment: In some cases, total RNA may copurify with genomic DNA from tissue samples. To remove copurified total RNA, perform an RNase treatment. Add 5µl of RNase A (Cat.# A7973) per milliliter of Elution Buffer (to be used in Section 6.A, Step 10, or Section 6.B, Step 9).

5. Maxwell[®] 16 Cartridge Preparation

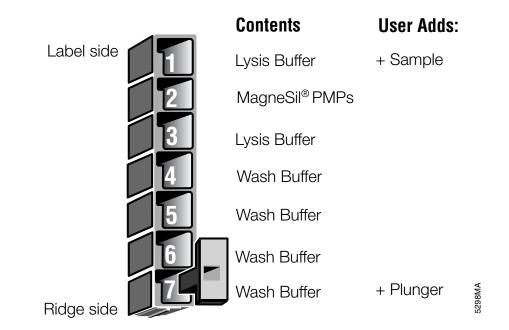
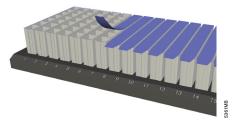


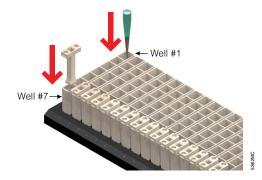
Figure 1. Maxwell® 16 DNA Purification Cartridge. This figure shows the contents of a cartridge for the Maxwell® 16 Blood, Cell or Tissue DNA Purification Kit. In all cases, a sample is added to well #1.



1. Place each cartridge to be used into the holder with the ridged side of the cartridge facing toward the numbered side of the rack. Remove the seal from each cartridge.



5. Maxwell[®] 16 Cartridge Preparation (continued)



Place one plunger into well #7 of each cartridge such that the bottom of the plunger is at the bottom of the cartridge. (Well #7 is the well closest to the ridged side of the cartridge.)
 Note: The plunger will fit loosely in the cartridge.



3.

Transfer your sample into well #1. (Well #1 is the well closest to the cartridge label and furthest from the user.) The Maxwell® 16 reagent 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.

The Maxwell[®] 16 reagent cartridges contain potentially hazardous chemicals. Wear gloves or other protective means when handling the reagent cartridges. Follow your institutional guidelines for disposal.

6. Automated DNA Purification on the Maxwell® 16 Instrument

6.A. Maxwell® 16 MDx Instrument (Cat.# AS3000)

Refer to the *Maxwell® 16 MDx Instrument Technical Manual* #TM320 for detailed information about setting up and running the Maxwell® 16 MDx Instrument.

- 1. Turn on the Maxwell[®] 16 MDx Instrument. The instrument will power up, display the firmware version number, proceed through a self-check and home all moving parts.
- 2. Verify that the Home screen indicates "SEV" and the SEV hardware is present. Press Run/Stop to continue.
- 3. Enter user and PIN, if this option is enabled.
- 4. Select DNA to access the protocols for Blood and Cells, Buffy Coat or Tissue DNA. Select the protocol required.
- 5. On the next screen, verify that the correct method and user were chosen. Select **Run/Stop** to continue.
- 6. Open the door when prompted on the screen, then select Run/Stop.



Warning: Pinch point hazard.

 Follow the instructions for bar code reader input in the Maxwell® 16 MDx Instrument Technical Manual #TM320 if this option is enabled.

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8. Transfer cartridges containing samples and plungers from the cartridge preparation rack onto the Maxwell[®] 16 platform. Ensure that the cartridges are placed into the instrument with the ridged side of the cartridge closest to the door.

Notes:

- · If you have difficulty fitting the cartridge in the platform, check the cartridge orientation.
- Insert the cartridge by first inserting the ridged side, then pressing down on the back of the cartridge to "click" it into place.
- If you are processing less than 16 samples, center the reagent cartridges on the platform, spacing them evenly outwards from the center.
- 9. Place one blue elution tube for each cartridge into the elution tube slots at the front of the platform.
- 10. Add 300µl of elution buffer to each blue elution tube.
- 11. Press the **Run/Stop** button. The platform will retract. Close the door.



Warning: Pinch point hazard.

The Maxwell[®] 16 MDx Instrument will immediately begin the purification run. The screen will display the approximate time remaining in the run.

Notes:

- a. Pressing the **Run/Stop** button or opening the door will pause the run.
- b. If the run is abandoned before completion, the instrument will wash the particles off the plungers and eject the plungers into well #7 of the cartridge. The samples will be lost.
- 12. When the automated purification run is complete, follow instructions for data transfer in the Maxwell® 16 MDx Instrument Technical Manual #TM320 and Maxwell® Sample Track Software Technical Manual #TM314.
- 13. Follow on-screen instructions at the end of the method to open door. Verify that plungers are located in well #7 of the cartridge at the end of the run. If plungers were not removed from the magnetic plunger bar, push them down gently by hand to remove them.
- 14. Press **Run/Stop** to extend the platform out of the instrument.



Warning: Pinch point hazard.

15. Remove the elution tubes from the heated elution tube slots, and place them into the Magnetic Elution Tube Rack. Transfer the eluted samples into storage tubes by pipetting. Discard the blue elution tubes after transfer of the eluted sample.

Note: To avoid particle transfer, use a pipet tip to aspirate samples away from the captured particles on the side of the blue elution tube.



Remove cartridges and plungers from the instrument platform and discard.



Do not reuse reagent cartridges, plungers or elution tubes.

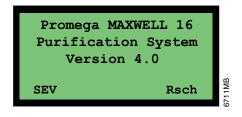
If you have configured your instrument to perform a UV light treatment, ensure samples are removed from the Maxwell® 16 MDx Instrument before UV light treatment to avoid damage to the nucleic acid.



6.B. Maxwell® 16 Instrument (Cat.# AS2000)

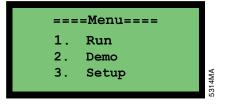
To use the Maxwell[®] 16 DNA Purification Kits (Cat.# AS1010 and AS1030), the Maxwell[®] 16 Instrument must be configured with the Maxwell[®] 16 SEV Hardware Kit (Cat.# AS1200). Reconfiguring the instrument is simple.

 Verify that the instrument mode is set to Research. Do this by closing the door and turning the Maxwell[®] 16 Instrument off, then on again. The instrument will power up and display the firmware version number, current operational mode and hardware configuration settings. Verify that "Rsch" and "SEV" are displayed as shown. If these settings are not displayed, refer to the instrument Technical Manual (TM295) for instructions on how to reset the instrument.



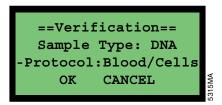
2. Use the Scroll Up or Scroll Down button to move the cursor to "Run" to perform a purification run. Press **Run/Stop** to select.

Note: "Demo" is an abbreviated purification run for demonstration purposes. "Setup" is used only to change the run mode of the instrument, which is not required for this procedure.



- 3. Use the Scroll Up or Scroll Down button to move the cursor to "DNA". Press Run/Stop to select.
- Use the Scroll Up or Scroll Down button to move the cursor to the purification method/sample type. Press Run/Stop to select.

 Verify that you have selected the correct protocol. Use the Scroll Up or Scroll Down button to move the cursor to OK. Press the Run/Stop button to continue with a purification run. Select Cancel if the information displayed is not correct.



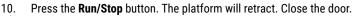
6. Open the door when prompted to do so on the LCD display. Press the **Run/Stop** button to extend the platform out of the instrument for easy insertion of the cartridges.



Warning: Pinch point hazard.

Transfer cartridges containing samples and plungers from the cartridge preparation rack onto the Maxwell[®] 16 platform. Ensure that the cartridges are placed into the instrument with the ridged side of the cartridge closest to the door.

- Notes:
- If you have difficulty fitting the cartridge in the platform, check the cartridge orientation.
- Insert the cartridge by first inserting the ridged side, then pressing down on the back of the cartridge to "click" it into place.
- If you are processing less than 16 samples, center the reagent cartridges on the platform, spacing them evenly
 outwards from the center.
- 8. Place one blue Elution Tube for each cartridge into the Elution Tube slots at the front of the platform.
- 9. Add 300µl of Elution Buffer to each blue Elution Tube.





Warning: Pinch point hazard.

11. The Maxwell[®] 16 Instrument will begin the purification run. The LCD screen displays the steps performed and approximate time remaining in the run.

Notes:

- Pressing the **Run/Stop** button or opening the door will pause the run. Close the door, if open, and select whether to **continue** or **terminate** the run.
- If you select to **terminate** the run before completion, the instrument will wash the particles off the plungers and remove the plungers into well #7 of the cartridge, and **your sample will be lost**.
- For instructions on recovering sample after a temporary power outage, please see the Maxwell[®] 16 Instrument Technical Manual.



6.B. Maxwell® 16 Instrument (Cat.# AS2000; continued)

- 12. When purification is complete, the LCD screen will display a message that the method has ended. Upon completion, open the instrument door. Check to make sure that all plungers have been removed from the magnetic rod assembly. If the plungers have not been removed, push them down gently by hand to remove them.
- 13. Press the Run/Stop button to extend the platform out from inside the instrument.
- 14. Remove the Elution Tubes from the platform-heated Elution Tube slots, and place them into the Magnetic Elution Tube Rack. Allow the residual magnetic particles to collect on the magnetized side of the tube. The amount of particles will vary with sample size and composition. Transfer the eluted samples into the storage tube by pipetting.



Note: To avoid particle transfer, use a pipette tip to aspirate samples away from the captured particles on the side of the blue Elution Tube.



 Remove cartridges and plungers from the instrument platform, and discard them. Do not reuse reagent cartridges, plungers or Elution Tubes.

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. E-mail: techserv@promega.com

Symptoms	Possible Causes and Comments		
Lower than expected A ₂₆₀	General		
(lower than expected yield)	Too much sample was processed. Processing more than the recommended maximum amounts of sample will not necessarily provide increased yields. Exceeding sample size limits may result in suboptimal DNA yield and purity.		
	Tissue Tissues that have undergone multiple freeze-thaw cycles may contain degraded DNA. Use fresh tissue samples whenever possible or avoid multiple freeze-thaw cycles of tissue.		
	Tissue sample was not efficiently ground by the instrument, resulting in inefficient sample lysis.Grinding and lysis of large chunks of tissue by the instrument can be improved by slicing or cutting a larger tissue chunk into smaller pieces and adding the smaller pieces to the first well of the DNA purification cartridge.		
	Blood Blood sample had a 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.		
No yield	Sample was placed into well #7 instead of well #1 of the DNA purification cartridge. Ensure that you have properly oriented the DNA purification cartridge so that you are adding the sample to well #1. Well #1 is the well closest to the labeled side of the cartridge.		
RNA contamination	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.		
Carryover of particles	Blood Samples with high white blood cell counts can become viscous and difficult to clear during elution. Perform a second particle capture using the Magnetic Elution Rack or the MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5342).		

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9. Related Products

Product	Size	Cat.#
Maxwell® 16 SEV Hardware Kit	1 each	AS1200
Maxwell® 16 LEV Hardware Kit	1 each	AS1250
Maxwell® 16 FFPE Tissue LEV DNA Purification Kit	48 preps	AS1130
Maxwell® 16 FFPE Plus LEV DNA Purification Kit	48 preps	AS1135
Maxwell® 16 LEV Blood DNA Kit	48 preps	AS1290
RNase A Solution, 4mg/ml	1ml	A7973

10. Summary of Changes

The following changes were made to the 2/25 revision of this document:

- 1. Removed discontinued Maxwell® 16 Cell DNA Purification Kit (Cat.# AS1020).
- 2 Deleted Section 4 and renumbered subsequent sections.
- 3. Updated Section 9, the cover page and document font.
- 4. Made minor text edits.
- 5. Removed the disclaimer.



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