

Technical Manual

Maxwell[®] 16

Blood DNA Purification System



In Vitro
Diagnostic
Medical Device



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INSTRUCTIONS FOR
USE OF PRODUCT
AS1015



Maxwell® 16

Blood DNA Purification System

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

1. Maxwell® 16 Blood DNA Purification System Intended Use	1
2. Product Components, Storage Conditions and Symbol Key	4
3. Before You Begin	6
A. Sample Preparation for Whole Blood Samples	6
B. Sample Preparation of Human Buffy Coat Samples	7
C. Maxwell® 16 Cartridge Preparation	8
4. Automated DNA Purification on the Maxwell® 16 Instrument	10
A. Maxwell® 16 IVD Instrument (AS3050-SC)	10
B. Maxwell® 16 Clinical Instrument (AS2050)	12
5. Troubleshooting	17

1. Maxwell® 16 Blood DNA Purification Kit Intended Use

The Maxwell® 16 Blood DNA Purification System^(a) (Cat.# AS1015) is intended for use with the Maxwell® 16 Instrument^(b) (Cat.# AS2050, AS3050-SC) 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 System 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 System is not intended for use as part of a specific in vitro diagnostic test.

The Maxwell® 16 Blood DNA Purification System is for professional use only. Diagnostic results obtained using DNA purified with this system must be interpreted in conjunction with other clinical or laboratory data.

Product Use Limitations

The Maxwell® 16 Blood DNA Purification System (Cat.# AS1015) is not intended for use with tissue samples or samples from body fluids other than blood. It is not intended for use with non-human samples, or for purification of RNA.

The Maxwell® 16 Blood DNA Purification System performance has been evaluated by isolating DNA from 300µl whole blood samples, or 250µl buffy coat samples, obtained from healthy individuals with a white blood cell count ranging from 4.2×10^6 to 1.2×10^7 .

The user is responsible for establishing performance characteristics necessary for downstream diagnostic applications. Appropriate controls must be included in any downstream diagnostic applications using DNA purified using the Maxwell® 16 Blood DNA Purification System.

Compliance with EU Directive 98/79/EC on in vitro diagnostic medical devices has been demonstrated for, and only applies to, use of the Maxwell® 16 Blood DNA Purification System (Cat.# AS1015) with the Maxwell® 16 Instrument (Cat.# AS2050, AS3050-SC) in the clinical/IVD mode.

Maxwell® 16 Blood DNA Purification Process

When used in conjunction with the Maxwell® 16 Instrument, the Maxwell® 16 Blood DNA Purification System (Cat.# AS1015), automates nucleic acid purification from up to 16 samples using cell lysis and binding of magnetized silica particles to nucleic acid as the primary separation principle.

The automated steps performed by the Maxwell® 16 System include:

- Sample lysis in the presence of a chaotropic agent and detergent.
- Binding of nucleic acids to magnetized silica particles.
- Washing of the bound particles away from other cellular components.
- Elution of nucleic acids into a formulation that can be added directly to standard PCR.

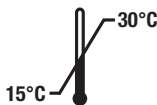
The user selects the appropriate processing protocol as prompted on the Maxwell® 16 instrument, places samples into the reagent cartridges, places the cartridges onto the Maxwell® 16 instrument platform and closes the door. The user then starts the instrument, which automatically performs all the steps in the protocol.

The temperature of samples is regulated by a heating system, which is controlled by the protocol. The extracted DNA can be used for PCR amplification.

2. Product Components, Storage Conditions and Symbol Key

Product	Size	Cat.#
Maxwell® 16 Blood DNA Purification System	48 preps	AS1015

Sufficient for 48 automated isolations.



Includes:

- 48 Maxwell® 16 Blood DNA Cartridges
- 50 Purification Plungers
- 50 Elution Tubes
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® 16 DNA Purification System at 15–30°C. See expiration date on the product label. Do not use product after the expiration date.



Safety Information: The reagent cartridges contain guanidine hydrochloride and guanidine thiocyanate, which are harmful and irritants. The cartridges also contain ethanol and isopropanol, which are flammable.



The Maxwell® 16 reagent cartridges are designed to be used with potentially infectious substances. Users should wear the appropriate protection (e.g., gloves and goggles) when handling infectious substances. Users should adhere to their institutional guidelines for the handling and disposal of all infectious substances when used with this system.



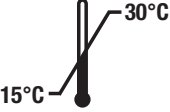










The Maxwell® 16 reagent cartridges contain potentially hazardous chemicals. Users should wear gloves when handling the reagent cartridges. Users should follow their institutional guidelines for disposal.

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



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Symbol Key

Symbol	Explanation	Symbol	Explanation
	In Vitro Diagnostic Medical Device		Authorized Representative
	Store at 15-30°C.		Manufacturer
	Important		Harmful. Irritant.
	Contains sufficient for "n" tests		Conformité Européenne
	Warning. Biohazard.		Warning. Pinch point hazard.
	Catalog number		Lot number
	Do not reuse		

3. Before You Begin

Materials to Be Supplied by the User

- pipets and pipet tips for sample transfer into prefilled reagent cartridges
- storage tubes for purified DNA samples

3.A. Sample Preparation for Whole Blood Samples

Table 1. Whole Blood Sample Volume and Preprocessing Requirements.

Sample Type	Kit	Volume	Preprocessing Requirements
Human whole blood	Blood DNA Purification System (Cat.# AS1015)	300µl	None

Whole Blood Sample Processing Capacity and Yield

The total yield of genomic DNA from whole blood samples depends on the sample volume and the number of white blood cells/ml. Each cartridge supplied in the Maxwell® 16 Blood DNA Purification System is designed for purification of genomic DNA from 300µl 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 cell number may adversely affect the yield and quality of the purified genomic DNA, and may cause cross-contamination of samples.

Notes:

1. Whole blood samples collected in EDTA, citrate or heparin-treated tubes can be used.
2. Blood samples should be stored at 4°C and processed within 7 days of collection.
3. When working with 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.

3.B. Sample Preparation of Human Buffy Coat Samples

Table 2. Buffy Coat Sample Volume and Preprocessing Requirements.


Sample Type	Kit	Volume	Preprocessing Requirements
Human buffy coat	Blood DNA Purification System (Cat.# AS1015)	250µl concentrated from 2.5ml whole blood	<ol style="list-style-type: none"> 1. Spin Vacutainer® tube for 20 minutes at 2,000 × g. 2. Harvest white cells using a 1ml pipet. 3. Add sample to well #1 .

Buffy Coat Sample Processing Capacity and Yield

Centrifugation of a whole blood sample at 2,000 × 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 pipet 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 in 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.

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

Notes:

1. 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.
2. When working with 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.
3. The concentration of the purified DNA should be measured by absorbance at A₂₆₀. DNA purity should be confirmed by agarose gel electrophoresis and by measuring the A₂₆₀/A₂₈₀ ratio, which is typically >1.7.
4. Appropriate controls must be included in downstream diagnostic applications using DNA purified with the Maxwell® 16 System.
5. During performance evaluation, carryover of DNA between cartridges was demonstrated to be <6pg/µl. The user is responsible for establishing performance characteristics necessary for downstream diagnostic applications.

3.C. Maxwell® 16 Cartridge Preparation

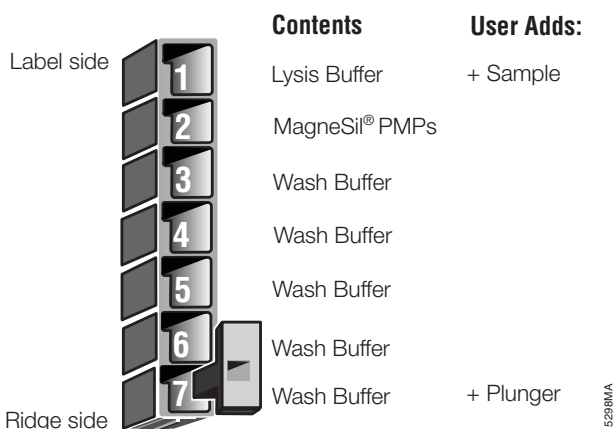
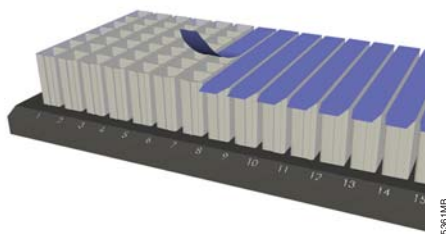


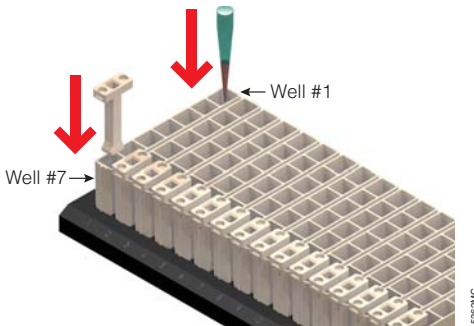
Figure 1. Maxwell® 16 Blood DNA Purification System Cartridge. The sample is added to well #1.



Follow standard laboratory procedures to avoid cross-contamination of samples. Wear gloves, and change them often. Use aerosol-resistant tips when transferring samples to minimize the potential for cross-contamination. Do not use cartridges if the seals are damaged or missing.



1. Place each cartridge to be used into the Maxwell® 16 Cartridge Rack with the ridged side of the cartridge facing toward the numbered side of the rack. Carefully remove the seal from each cartridge. Take care to avoid contamination of the reagents when removing the seal.



2. Place one plunger into well #7 of each cartridge, making sure 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.) Take care to avoid contamination of samples during transfer and to ensure that samples are correctly identified and tracked.



The Maxwell[®] 16 reagent cartridges are designed to be used with potentially infectious substances. Users should wear the appropriate protection (e.g., gloves and goggles) when handling infectious substances. Users should follow their 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. Users should wear gloves when handling the reagent cartridges. Users should follow their institutional guidelines for disposal.



The blue elution tubes will be used later in the set-up process.

4. Automated DNA Purification on the Maxwell® 16 Instrument

4.A. Maxwell® 16 IVD Instrument (AS3050-SC)

Refer to the *Maxwell® 16 IVD Instrument Technical Manual #TM315* for detailed information about setting up and running the Maxwell® 16 IVD Instrument.

1. Turn on the Maxwell® 16 IVD 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. At the Protocols screen, select “Blood” or “Buffy Coat”.
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.

7. Follow the instructions for bar code reader input in the *Maxwell® 16 IVD Instrument Technical Manual #TM315* if this option is enabled.
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 IVD Instrument will immediately begin the purification run. The screen will display the approximate time remaining in the run.

Notes:

1. Pressing the Run/Stop button or opening the door will pause the run.
 2. 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 IVD Instrument Technical Manual #TM315* 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..



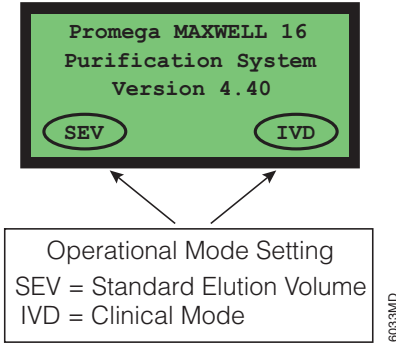
16. Remove cartridges and plungers from the instrument platform and discard.



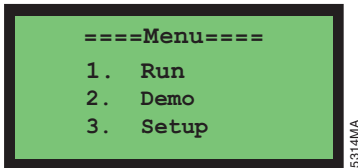
Do not reuse reagent cartridges, plungers or elution tubes.

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

4.B. Automated DNA Purification on the Maxwell® 16 Clinical Instrument (AS2050)



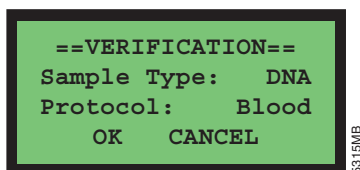
1. The Maxwell® 16 System is capable of running research and forensic methods as well as the clinical method. Before use, verify that the instrument is set to clinical mode. Do this by closing the door and turning the Maxwell® 16 Instrument off and then on again. The firmware version number and mode setting will be displayed as shown. If the screen is different from that shown above, refer to the *Maxwell® 16 Instrument Operating Manual (#TM300)* for instructions on how to reset the instrument to clinical mode.



2. To perform a purification run, use one of the scroll buttons to move the cursor to "Run". Press "Run/Stop" to select.

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

3. Use one of the scroll buttons to move the cursor to the required protocol/sample type. Press "Run/Stop" to select.



4. Verify that you have selected the correct protocol. Use one of the scroll buttons 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.

5. 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.



6. 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.

7. Place one blue elution tube for each cartridge into the elution tube slots at the front of the platform.
8. Add 300µl of elution buffer to each blue elution tube.

4.B. Automated DNA Purification on the Maxwell® 16 Clinical Instrument (AS2050) (continued)



9. Press the “Run/Stop” button. The platform will retract. Close the door.

Warning: Pinch point hazard.

10. The Maxwell® 16 Instrument will begin the purification run. The LCD screen displays the steps performed and the 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 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 troubleshooting section of the *Maxwell® 16 Instrument Operating Manual* (#TM300).

11. 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 the 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.

12. Press the “Run/Stop” button to extend the platform out from the instrument.
13. 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.



14. Remove cartridges and plungers from the instrument platform and discard. Do **not** reuse reagent cartridges, 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	Causes and Comments
Lower than expected A_{260} (lower than expected yield)	<p>Whole 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.</p> <hr/> <p>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.</p> <hr/> <p>Too much sample was processed. Processing more than the recommended amount of sample will not necessarily increase yields. Exceeding the sample size limit may result in suboptimal yield and purity of the DNA.</p>
No yield	<p>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.</p>
Carryover of particles	<p>DNA purified from 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 remove the particles by centrifugation.</p>

©U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Australian Pat. No. 732756, European Pat. No. 1 204 741, Mexican Pat. No. 209436 and other patents pending..

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