

TECHNICAL MANUAL

Maxwell[®] 16 FFPE Plus LEV DNA Purification Kit

Instructions for Use of Product
AS1135

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



Maxwell[®] 16 FFPE Plus LEV DNA Purification 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

Pathologists have long used formalin-fixed, paraffin-embedded (FFPE) tissue samples to examine morphology. With the advent of PCR amplification, these samples provide the potential to correlate morphology with genotype. However, tissue fixation can result in cross-linking between proteins and DNA and the potential for PCR inhibition.

The Maxwell[®] 16 FFPE Plus LEV DNA Purification Kit^(a) is used with the Maxwell[®] 16 Instrument configured with the low elution volume (LEV) hardware (Cat. # AS2000 or AS3000), and is specifically designed for purification of DNA from one to ten 5µm thin sections of FFPE tissue. The purified DNA is suitable for amplification applications including qPCR. However, tissue that has been stored in formalin for extended periods of time may be extensively cross-linked and/or too degraded to extract amplifiable DNA.



1. Description (continued)

The Maxwell® 16 FFPE Plus LEV DNA Purification Kit provides an easy method for efficient, automated purification of genomic DNA from FFPE tissue sections. The Maxwell® 16 Instrument is supplied with preprogrammed purification procedures and is designed for use with the prefilled reagent cartridges, maximizing simplicity and convenience. The instrument can process up to 16 samples in 30 minutes following an overnight Proteinase K digestion.

The Maxwell® 16 Instrument, together with customized cartridges, purifies DNA using silica-clad paramagnetic particles (PMPs), which provide a mobile solid phase that optimizes capture, washing and elution of the target material. The Maxwell® 16 Instrument is a magnetic particle-handling instrument that efficiently processes liquid samples, transports the PMPs through purification reagents in the prefilled cartridges (Figure 1), and mixes during processing. The paramagnetic particle-based methodology avoids common problems such as clogged tips or partial reagent transfers, which result in suboptimal purification processing by other commonly used automated systems.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® 16 FFPE Plus LEV DNA Purification Kit	48 preps	AS1135

For Laboratory Use. Sufficient for 48 automated isolations from formalin-fixed, paraffin-embedded tissue samples. Includes:

- 48 Maxwell® 16 LEV Cartridge (MCG)
- 50 LEV Plungers
- 50 Elution Tubes, 0.5ml
- 25ml Nuclease-Free Water
- 32ml Lysis Buffer
- 15ml Incubation Buffer
- 2 Proteinase K Tubes

Storage Conditions: Store the Maxwell® 16 FFPE Plus LEV DNA Purification Kit at 15–30°C. Upon receipt, store the Proteinase K tubes at –20°C. Store the prepared Proteinase K solution at –20°C.

Safety Information: The reagent cartridges contain ethanol, isopropanol and guanidine thiocyanate. These substances should be considered flammable, harmful and irritants. Wear gloves or other protective means. Wipe up any spills immediately, and clean the instrument after each use. For instrument cleaning instructions, refer to the Technical Manual supplied with your Maxwell® 16 Instrument.

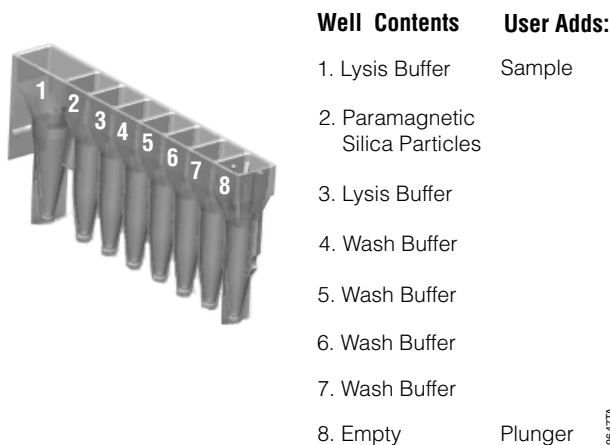


Figure 1. Maxwell® 16 LEV Cartridge (MCG).

3. Maxwell® 16 Instrument Hardware and Firmware Setup

To use the Maxwell® 16 FFPE Plus LEV DNA Purification Kit, the Maxwell® 16 Instrument must be configured with LEV hardware. If your Maxwell® 16 Instrument contains standard elution volume (SEV) hardware, it will need to be reconfigured using the Maxwell® 16 LEV Hardware Kit (Cat. # AS1250). Reconfiguring the instrument is simple and easy. For instructions to properly set up your instrument, refer to the Technical Manual supplied with your Maxwell® 16 Instrument.

4. Sample Preprocessing

To maximize the amount of DNA purified from FFPE tissues, use the protocol in Section 4.B. The protocol included in this section involves a Proteinase K treatment step, which has been demonstrated to increase the yield from a variety of FFPE sample types.

The protocol below uses the Incubation Buffer for the Proteinase K digestion. If another Proteinase K digestion buffer is used, keep the concentration of SDS below 0.5% or a precipitate will form when the Lysis Buffer is added.



4.A. Preparation of Stock Proteinase K Solution for Sample Preprocessing

1. Add 500 μ l of Nuclease-Free Water to each tube of lyophilized Proteinase K, and gently swirl to dissolve. The final concentration of Proteinase K will be 20mg/ml.
2. Dispense the Proteinase K solution into smaller aliquots that reflect usage, and store at -20°C for up to 1 year. The Proteinase K can be frozen and thawed up to five times with no significant loss in activity. Prior to use, Proteinase K should be thawed and stored on ice.

4.B. Sample Preprocessing Protocol—FFPE Tissues

Materials to Be Supplied by the User

- 70°C heat block or water bath
- Microtubes, 1.5ml (Cat.# V1231)
- aerosol-resistant micropipette tips
- FFPE tissue sample

Prepare a stock 20mg/ml Proteinase K solution (see Section 4.A).

1. Scrape 1–10 \times 5 μm thin section(s) from the FFPE tissue sample into a single microtube.
2. Centrifuge the samples briefly at full speed to collect the sample at the bottom of the tube. Overlay the samples with 20 μ l of Proteinase K solution and 180 μ l of Incubation Buffer.
3. Close the tube cap, and incubate the sample at 70°C overnight.
4. Add two volumes of Lysis Buffer to each sample (e.g., if 200 μ l of Incubation Buffer/Proteinase K solution was added to the solid substrate, add 400 μ l of Lysis Buffer).

Note: The maximum volume that can be processed by the LEV RNA Cartridge is 750 μ l.

5. Vortex sample and Lysis Buffer briefly.
6. Close the lid of the Microtube and save until ready for automated DNA extraction using the Maxwell[®] 16 LEV Instrument (Section 5).

Note: Do not refrigerate or freeze sample. Leave preprocessed sample at room temperature overnight, if necessary.

5. Maxwell® 16 Automated DNA Purification

5.A. Preparation of Samples for Maxwell® 16 LEV Cartridges

1. Change gloves before handling cartridges, LEV Plungers and Elution Tubes. Place the cartridges to be used in the Maxwell® 16 LEV Cartridge Rack (Cat. # AS1251). Place each cartridge in the rack with the label side 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.

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

Notes:

1. If you are processing fewer than 16 samples, center the cartridges on the platform.
2. Specimen or reagent spills on any part of the Maxwell® 16 LEV Cartridge Rack should be cleaned with a detergent-water solution, followed by 70% ethanol, then water. Do not use bleach on any instrument parts.
2. Place an LEV Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
3. Place Elution Tubes in the front of the Maxwell® 16 LEV Cartridge Rack. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube.



Notes:

1. An elution volume of 50µl is recommended. Elution volumes less than 50µl may result in lower yield. For more concentrated DNA, an elution volume of 30µl may be used, but the total yield may be affected. Do not use elution volumes less than 30µl. Elution volumes of greater than 100µl may result in DNA too dilute for some downstream assays.
2. Ensure that the Nuclease-Free Water is in the bottom of the tube. If the Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
3. Use only the Elution Tubes provided with the kit; other tubes may not work with the Maxwell® 16 Instrument.

5.A. Preparation of Samples for Maxwell® 16 LEV Cartridges (continued)

4. Transfer the sample to well #1 of the cartridge. Well #1 is the well closest to the cartridge label and furthest from the Elution Tube.



5. Proceed to Section 5.B for Cat.# AS3000 instruments and Section 5.C for Cat.# AS1000 and AS2000 instruments.

5.B. Setup for AS3000 Maxwell® 16 MDx Instrument LEV

Refer to the *Maxwell® 16 MDx Instrument Technical Manual #TM320* for detailed information.

To run this protocol, you must have Maxwell® 16 firmware version 1.2 or higher installed on your 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 “LEV” and the LEV hardware is present. Press “Run” to continue.
3. Enter user and PIN, if this option is enabled.
4. Select “DNA” at the menu screen.
5. Select “FFPE/Cells” at the protocol screen.
6. On the next screen, verify that the correct method and user were chosen. Select “Run/Stop” to continue.
7. Open the door when prompted on the screen, then select “Run/Stop”.



Warning: Pinch point hazard.

8. Follow on-screen instructions for bar code reader input if this option is enabled.

9. Transfer the Maxwell® 16 LEV Cartridge Rack containing the prepared cartridges on the Maxwell® 16 Instrument platform. Ensure that the rack is placed in the Maxwell® 16 Instrument with the Elution Tubes closest to the door. The rack will only fit in the instrument in this orientation. If you have difficulty fitting the rack on the platform, check that the rack is in the correct orientation. Ensure the rack is level on the instrument platform.
Note: Hold the Maxwell® 16 LEV Cartridge Rack by the sides to avoid dislodging cartridges from the rack.
10. Verify that samples were added to well #1 of the cartridges, cartridges in the rack are loaded on the instrument, Elution Tubes are present with 50µl of Nuclease-Free Water and LEV Plungers are in well #8.
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:

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 #8 of the cartridge. The samples will be lost.
12. When the automated purification run is complete, follow instructions on the screen for data transfer. For detailed instructions, refer to the *Maxwell® 16 MDx Instrument Technical Manual #TM320* and *Maxwell® Sample Track Software Technical Manual #TM314*.

End of Run

13. Follow on-screen instructions at the end of the method to open 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 magnetic plunger bar, push them down gently by hand to remove them.
14. Press the Run/Stop button to extend the platform out of the instrument.
15. Remove the Maxwell® 16 LEV Cartridge Rack from the instrument. Remove Elution Tubes containing DNA, and close the tubes.

Notes:

1. Small amounts of resin particles may be present in the Elution Tube. This will not affect downstream applications.
2. To prevent evaporation of eluted DNA, cap Elution Tubes within 15 minutes after completing the purification run.



Warning: Hot Surface. Burn Hazard.

5.B. Setup for AS3000 Maxwell® 16 MDx Instrument LEV (continued)

16. Determine that the purified DNA sample yield meets the input requirements for the appropriate downstream applications prior to use in those applications.

Note: Absorbance readings for purified FFPE samples may overestimate yield; we recommend using other methods for determining yield (1).

17. Remove cartridges and plungers from the cartridge rack, and discard as hazardous waste. Do not reuse reagent cartridges, LEV Plungers or Elution Tubes.

For the Maxwell® 16 MDx Instrument, ensure samples are removed before the UV light treatment to avoid damage to the nucleic acid.

5.C. Setup for AS1000 and AS2000 Maxwell® 16 Instruments

Refer to the *Maxwell® 16 Instrument Operating Manual #TM274* for AS1000 instrument or *Maxwell® 16 Instrument Operating Manual #TM295* for AS2000 instrument for detailed information.

To run this protocol, you must have Maxwell® 16 firmware version 4.71 or higher installed on your instrument.

1. Turn on the Maxwell® 16 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 instrument settings indicate an “LEV” hardware configuration and “Rsch” operational mode setting.
3. Select “Run” on the Menu screen, and press the Run/Stop button to start the method.
4. Select “DNA” on the Menu screen.
5. Select “FFPE/Cells” at the protocol screen.
6. Open the door when prompted to do so on the screen. Press the Run/Stop button to extend the platform.



Warning: Pinch point hazard.

7. Transfer the Maxwell® 16 LEV Cartridge Rack containing the prepared cartridges on the Maxwell® 16 Instrument platform. Ensure that the rack is placed in the Maxwell® 16 Instrument with the Elution Tubes closest to the door. The rack will only fit in the instrument in this orientation. If you have difficulty fitting the rack on the platform, check that the rack is in the correct orientation. Ensure that the cartridge rack is level on the instrument platform.

Note: Hold the Maxwell® 16 LEV Cartridge Rack by the sides to avoid dislodging cartridges from the rack.

8. Verify that samples were added to well #1 of the cartridges, cartridges in the rack are loaded on the instrument, Elution Tubes are present with 50µl of Nuclease-Free Water and LEV Plungers are in well #8.
9. Press the Run/Stop button. The platform will retract. Close the door.



Warning: Pinch point hazard.

10. The Maxwell® 16 Instrument will immediately begin the purification run. The screen will display the steps performed and 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 #8 of the cartridge. The sample will be lost.
11. When the automated purification run is complete, the LCD screen will display a message that the method has ended.

End of Run

12. Follow on-screen instructions at the end of the method to open 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 magnetic plunger bar, push them down gently by hand to remove them.
13. Press the Run/Stop button to extend the platform out of the instrument.
14. Remove the Maxwell® 16 LEV Cartridge Rack from the instrument. Remove Elution Tubes containing DNA, and close the tubes.

Notes:

1. Small amounts of resin particles may be present in the Elution Tube. This will not affect downstream applications.
2. To prevent evaporation of eluted DNA, cap Elution Tubes within 15 minutes after completing the purification run.



Warning: Hot Surface. Burn Hazard.

15. Remove the cartridges and plungers from the Maxwell® 16 LEV Cartridge Rack, and discard as hazardous waste. Do not reuse reagent cartridges, LEV Plungers or Elution Tubes.



6. 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
Low DNA concentration	Insufficient sample was processed: <ul style="list-style-type: none">• Add more starting material for preprocessing to increase yield.• Optimize the preprocessing incubation temperature to improve final DNA concentration.
Poor PCR results	Too much starting material. Reduce the amount of sample used for purification. Confirm concentration using a quantitation method. Wrong elution buffer was added. Use only the Nuclease-Free Water supplied with the Maxwell® 16 FFPE Plus LEV DNA Purification Kit. Poor-quality FFPE samples. Fixation conditions, such as prolonged storage in fixative, can affect PCR performance. Buffered formalin prevents acidification of tissue during fixation.
Instrument calibration error	Verify nothing is physically blocking the movement of the platform, plunger bar or magnetic rod assembly. Turn the machine off and then on to cycle the power. The instrument will rehome itself. If the calibration error occurs again after power cycling, please contact Promega for service. After cycling power, run a “Demo” method without any cartridges in the machine. If another calibration error occurs during the “Demo” run, please contact Promega for service. Ensure the Maxwell® 16 LEV Hardware Kit (Cat.# AS1250) is installed on your instrument. The cartridges are not completely seated on the platform. Ensure the cartridges are pressed firmly into place and that the tray is installed correctly on the platform.

Symptoms	Causes and Comments
Instrument calibration error (continued)	Incorrect elution tubes used with the system. To prevent a Z-axis collision, only use the 0.5ml Elution tubes provided with the FFPE Plus LEV DNA Purification Kit. Other tubes may have different dimensions.
PMPs carryover during elution	A small amount of particles are visible in elution tube. The presence of particles in the elution tube will not affect the final DNA concentration or downstream applications. If desired, an additional particle capture step may be performed using the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341) or Maxwell® 16 LEV Magnet (Cat.# AS1261).

7. Related Products

Instrument Hardware Accessories

Product	Cat.#
Maxwell® 16 LEV Hardware Kit	AS1250
Maxwell® 16 LEV Cartridge Rack	AS1251
Maxwell® 16 SEV Hardware Kit	AS1200
Maxwell® 16 Cartridge Rack (for use with standard configuration)	AS1201
Maxwell® 16 Magnetic Elution Rack (for use with standard configuration)	AS1202
Maxwell® 16 LEV Magnet	AS1261

Low Elution Volume (LEV) Kits

Product	Size	Cat.#
Maxwell® 16 FFPE Tissue LEV DNA Purification Kit	48 preps	AS1130
Maxwell® 16 Tissue LEV Total RNA Purification Kit	48 preps	AS1220
Maxwell® 16 Cell LEV Total RNA Purification Kit	48 preps	AS1225
Maxwell® 16 Cell LEV Total DNA Purification Kit	48 preps	AS1140
Maxwell® 16 Viral Total Nucleic Acid Purification Kit	48 preps	AS1150
Maxwell® 16 LEV Blood DNA Kit	48 preps	AS1290



7. Related Products (continued)

Standard Elution Volume (SEV) Kits

Product	Size	Cat.#
Maxwell® 16 Blood DNA Purification Kit	48 preps	AS1010
Maxwell® 16 Cell DNA Purification Kit	48 preps	AS1020
Maxwell® 16 Tissue DNA Purification Kit	48 preps	AS1030
Maxwell® 16 Mouse Tail DNA Purification Kit	48 preps	AS1120
Maxwell® 16 Total RNA Purification Kit	48 preps	AS1050
Maxwell® 16 Polyhistidine Protein Purification Kit	48 preps	AS1060

8. Reference

1. Bonin, S. *et al.* (2010) Multicentre validation study of nucleic acids extraction from FFPE tissues. *Virchows Arch.* **457**, 309–17.

9. Summary of Changes

The following changes were made to the 10/17 revision of this document:

1. The option was removed to incubate the sample for 1 hour instead of overnight.

^(a)U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, European Pat. Nos. 0895546, 1367137 and 1204741, Japanese Pat. Nos. 3253638 and 4425513.

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