



Promega

Technical Bulletin

Maxwell® 16 FFPE Tissue LEV DNA Purification Kit

INSTRUCTIONS FOR USE OF PRODUCT AS1130.

Note: Please ensure that all sealing tape and any residual adhesive are removed from the Maxwell® 16 System cartridges before placing the cartridges into the instrument.



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Maxwell[®] 16 FFPE Tissue LEV DNA Purification Kit

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 Bulletin. Please contact Promega Technical Services if you have questions on use
of this system. E-mail: 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 Tissue LEV (Low-Elution Volume) DNA Purification Kit^(a,b) is used with the Maxwell[®] 16 Instrument (Cat.# AS2000) and LEV Hardware Kit (Cat.# AS1250) and is specifically designed for optimal purification of DNA from one to ten sections (5µm) of FFPE tissue samples. To help minimize PCR inhibitor carryover, small amounts of DNA-binding paramagnetic particles are used, and as a result the DNA-binding capacity of the system is limited to a few hundred nanograms of pure DNA 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.

The Maxwell[®] 16 FFPE Tissue 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

1. Description (continued)

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 magnetic 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 Tissue LEV DNA Purification Kit	48 preps	AS1130

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

- 48 Maxwell® 16 LEV Cartridge (MCB)
- 50 LEV Plungers
- 50 Elution Tubes, 0.5ml
- 20ml Elution Buffer
- 32ml Lysis Buffer
- 15ml Incubation Buffer
- 2 Proteinase K Tubes

Storage Conditions: Store the Maxwell® 16 FFPE 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.

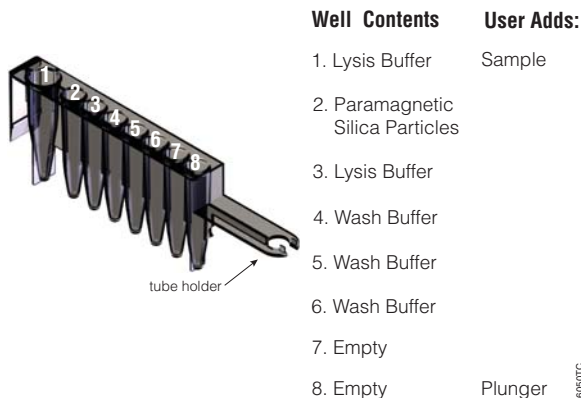


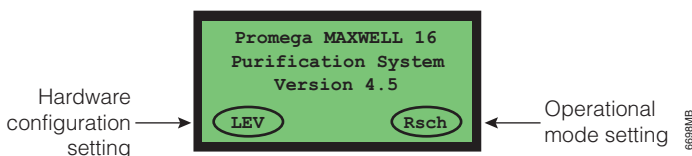
Figure 1. Maxwell® 16 LEV Cartridge (MCB).

3. Maxwell® 16 Instrument Hardware and Firmware Setup

Users with a Maxwell® 16 Instrument need to configure their instrument using the Maxwell® 16 LEV Hardware Kit (Cat.# AS1250). Reconfiguring the instrument is simple and easy. For instructions to properly set up the instrument, please refer to the *Maxwell® 16 Instrument Technical Manual*.

The first time the Maxwell® 16 Instrument is powered up, a series of user prompts will appear on the Navigation LCD. The Maxwell® 16 FPPE Tissue LEV DNA Purification Kit is intended to be used with the LEV settings and the “RsCh” (Research) method on the instrument. Once the “RsCh” method is set up on the instrument, all subsequent power-ups of the instrument will automatically default to these settings.

After completing the firmware setup, the LCD should display the following screen:



Note: Your instrument should display Version 4.5 or higher.

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)
- Nuclease-Free Water (Cat.# P1193)
- aerosol-resistant micropipette tips

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

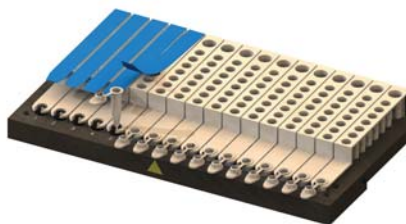
1. Scrape one to ten 5 μ m sections from the FFPE sample of interest into a single microtube.
2. Centrifuge the samples briefly at full speed to collect the sample at the bottom of the tube. Overlay the samples with 20 μ l of Proteinase K solution and 180 μ l of Incubation Buffer.
3. Close the tube cap, 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 DNA 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



1. Place the number of cartridges to be used into the Maxwell® 16 LEV Cartridge Rack (Cat.# AS1251). Place each cartridge into the rack with the tube holder (Figure 1) facing towards the numbered side of the rack. Hold the cartridge firmly and remove the seal.

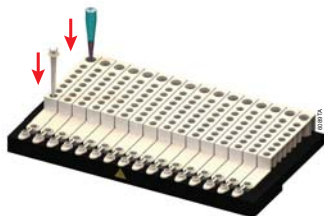
Note: Carefully peel back the plastic coating so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed from the Maxwell® 16 System cartridges before placing the cartridges into the instrument.

Alternatively, remove the seal first, then place each cartridge into the rack.

Notes:

- It is easiest to insert the cartridge by inserting the tube holder side first and then pressing down on the back of the cartridge to snap it into place.
- If you are processing fewer than 16 samples, center the reagent cartridges on the platform, spacing them evenly outwards from the center.

5. Maxwell® 16 Automated DNA Purification (continued)



2. Place one plunger into well #8 of each cartridge. (Well #8 is the well closest to the tube holder).

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 farthest from the user).



4. Place 0.5ml Elution Tubes into the elution tube holder at the front of the cartridge. Add 50µl of Elution Buffer to each Elution Tube. For more concentrated DNA, the volume can be decrease to as little as 25µl. Do not use less than 25µl of Elution Buffer.
5. Turn the Maxwell® 16 Instrument on. The instrument will power up, display the firmware version number, proceed through a self-check and home all axes.
6. Scroll to “Run” on the Menu screen and press the “Run/Stop” button to start the method prior to loading the Maxwell® 16 LEV Cartridge Rack.
7. Select “DNA” at the menu screen.
8. Select “FFPE/Cells” at the protocol screen
9. Open the door when prompted to do so on the LCD display. Press the “Run/Stop” button to extend the platform.



Warning. Pinch point hazard.

 Do not start the instrument prior to performing Step 4.

10. Transfer the Maxwell® 16 LEV Cartridge Rack onto the Maxwell® 16 platform. Ensure that the rack is placed into the Maxwell® 16 Instrument with the tube holders of the cartridges closest to the door. The rack will only fit into the instrument in this orientation. If you have difficulty fitting the rack onto the platform, check that the rack is in the correct orientation. Ensure the cartridge rack is completely level.
11. Ensure that the desired volume of Elution Buffer has been added to the Elution Tubes prior to starting the automated method.

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

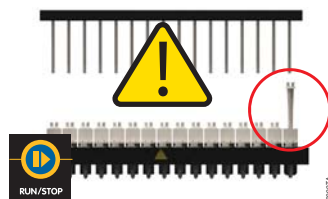


Warning. Pinch point hazard.

12. The Maxwell® 16 Instrument will immediately begin the purification run. The LCD screen will display 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 the program is terminated before completion, the instrument will wash the particles off the plungers and eject the plungers into well #8 of the cartridge.



13. When purification is complete, the LCD screen will display a message that the method has ended. Upon method completion, open the instrument door. The plungers should be located in well #8 at the end of the run. Check to make sure that all of 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 from the magnetic rod assembly.

5. Maxwell® 16 Automated DNA Purification (continued)

14. Press the “Run/Stop” button to extend the platform.
15. Remove the Elution Tubes from the heated elution tube slots, and close the top of each tube. Label each tube, and store the final extract until ready to use.

Notes:

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



Warning. Hot surface. Burn hazard.



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16. Remove cartridges and plungers from the instrument platform and discard. Do not reuse Reagent Cartridges, Plungers or Elution Tubes.
17. Use one of the scroll (up/down) buttons to move the cursor to select “Yes” or “No” to run the purification method again.

If “Yes” is selected, the Menu screen will appear.

If “No” is selected, the platform is retracted back into the instrument. You are then prompted to close the door.
18. A diagnostic axis check is automatically performed whether another run is chosen or not. If the check is successful, the LCD screen will display a message indicating so. If the check is unsuccessful, an error message will appear. Refer to Section 6 for information about resolving the error.

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	<p>Too much starting material. Reduce the amount of sample used for purification. Confirm concentration using a quantitation method.</p> <p>Wrong elution buffer was added. Use only the Elution Buffer supplied with the Maxwell® 16 FFPE Tissue LEV DNA Purification Kit.</p> <p>Poor quality FFPE samples. Fixation conditions, such as prolonged storage in fixative, can affect PCR performance. Buffered formalin prevents acidification of tissue during fixation.</p>
Instrument calibration error	<p>Verify nothing is physically blocking the movement of the platform, plunger bar or magnetic rod assembly.</p> <p>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.</p> <p>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.</p> <p>If using a Maxwell® 16 Instrument (Cat.# AS2000), ensure the Maxwell® 16 LEV Hardware Kit (Cat.# AS1250) is installed.</p> <p>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.</p>

6. Troubleshooting (continued)

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 Tissue LEV DNA Purification Kit. Other tubes may have different dimensions.
PMPs carryover during elution	A small amount of particle 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).

7. Related Products

Product	Size	Cat.#
Maxwell® 16 LEV Hardware Kit	1 each	AS1250
Maxwell® 16 LEV Cartridge Rack	1 each	AS1251
Maxwell® 16 Instrument*	1 each	AS2000
Proteinase K*	100mg	V3021
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	0.5ml	Z5341

*For Laboratory Use.

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©U.S. Pat. No. 6,673,631, European Pat. No. 1 204 741 and other patents pending.

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