TECHNICAL BULLETIN

Maxwell® 16 Cell LEV DNA Purification Kit

Instructions for Use of Product
AS1140

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

Revised 6/12
TB383

Maxwell® 16 Cell 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 Bulletin.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Research and clinical lab scientists often rely on limited numbers of cultured or primary mammalian cells to investigate various aspects of disease. The Maxwell® 16 Cell LEV DNA Purification Kit[a,b,c] is used with the Maxwell® 16 Instrument configured with the low elution volume (LEV) hardware (Cat.# AS2000-LX, AS2000-LF or AS3000-LC), and is specifically designed for automated purification of genomic DNA from less than 10,000 (10^4) cells (Figure 1). The binding capacity of the system is limited to a few hundred nanograms of highly pure DNA suitable for amplification applications. If sensitive qPCR methods are used for detection, DNA can be purified and amplified from as few as 10 cells (Table 1).

The Maxwell® 16 Instrument is supplied with preprogrammed purification procedures and is designed for use with predispensed reagent cartridges, maximizing simplicity and convenience. The instrument can process up to 16 samples in 30–45 minutes. The Maxwell® 16 Instrument purifies samples using silica-coated paramagnetic particles (PMPs), which provide a mobile solid phase that optimizes capture, washing and elution of the target material. The Maxwell® 16 is a magnetic particle-handling instrument that efficiently preprocesses liquid and solid samples, transports the PMPs through purification reagents in the prefilled cartridges (Figure 2), and mixes 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.

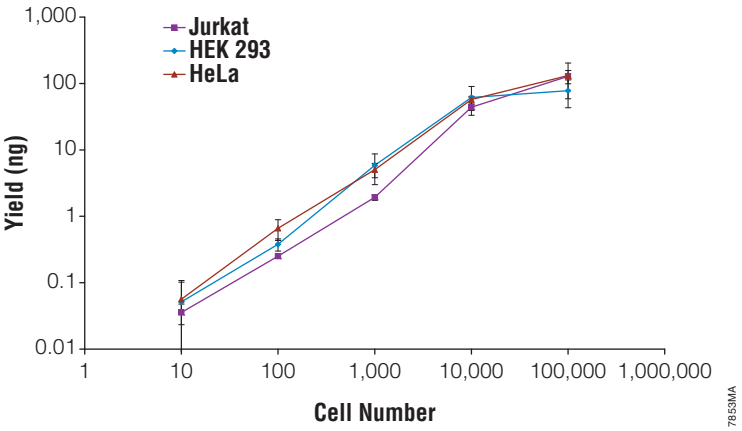

Figure 1. DNA yield from various cell numbers/types using the Maxwell® 16 Cell LEV DNA Purification Kit. Jurkat, HEK 293 and HeLa cells were suspended in 100µl of PBS and added directly to well #1 of the Maxwell® 16 LEV Cartridge. Isolated DNA was eluted in 50µl of Elution Buffer and quantitated with the Plexor® HY System (Cat.# DC1000) using a Stratagene Mx3005P® Quantitative PCR System.

Table 1. Appropriate Quantitation Methods for DNA Purified from 10, 100, 1,000 and 10,000 Cells Using the Maxwell® 16 Cell LEV DNA Purification Kit.

Number of Cells	Quantitation Method*		
	Real-Time qPCR	PicoGreen® Staining (Microplate format)	Absorbance A_{260}
10	+	–	–
100	+	–	–
1,000	+	+	–
10,000	+	+	–

*Quantitation was performed by real-time PCR (Plexor® HY System, Cat.# DC1000) or PicoGreen® staining. Absorbance measurements are unsuitable for quantitation of DNA yields from 10,000 cells or less.

Well	Contents	User Adds:
1	Lysis Buffer	Sample
2	Paramagnetic Silica Particles	
3	Lysis Buffer	
4	Wash Buffer	
5	Wash Buffer	
6	Wash Buffer	
7	Empty	
8	Empty	Plunger

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Figure 2. Maxwell® 16 LEV Cartridge (MCB).

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® 16 Cell LEV DNA Purification Kit	**48 preps**	**AS1140**

For Laboratory Use. Sufficient for 48 automated isolations. Includes:

- 48 Maxwell® 16 LEV Cartridges (MCB)
- 50 LEV Plungers
- 50 Elution Tubes, 0.5ml
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® 16 Cell LEV DNA Purification Kit at 15–30°C.

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

3. Maxwell® 16 Instrument Hardware and Firmware Setup

To use the Maxwell® 16 Cell 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, please refer to the *Maxwell® 16 MDx Instrument Technical Manual* #TM320 for Cat.# AS3000, *Maxwell® 16 Instrument Technical Manual* #TM295 for Cat.# AS2000 or *Maxwell® 16 Instrument Operating Manual* #TM274 for Cat.# AS1000 for directions.

4. Sample Preprocessing

Materials to Be Supplied by the User

- Microtubes, 1.5ml (Cat.# V1231) or larger
- PBS (10.1mM Na_2HPO_4, 1.8mM KH_2PO_4, 140mM NaCl, 2.7mM KCl [pH 7.3])

1. Pellet cells from the sample by centrifugation. For volumes less than 1.5ml, centrifuge at 13,000–16,000 × *g* for 1–2 minutes at room temperature. For larger volumes, centrifuge at 2,000 × *g* for 10 minutes.
2. Remove the supernatant, being careful not to disturb the cell pellet. If no pellet is visible, avoid the position in the tube where a pellet would be expected. Small cell numbers may result in a pellet that is not visible by eye.
3. Resuspend the pellet in up to 100µl of PBS.

5. Maxwell® 16 Automated DNA Purification

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

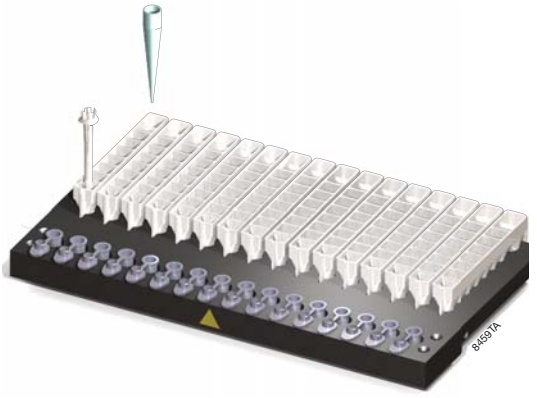
1. Change gloves before handling cartridges, LEV Plungers and Elution Tubes. Place the cartridges to be used in the Maxwell® 16 LEV Cartridge Rack (Cat.# AS1251). Place each cartridge in the rack with the label side facing away from the Elution Tubes. Press down on the cartridge to snap it into position. 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.

   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 Elution Buffer to the bottom of each Elution Tube. For more concentrated DNA, the volume can be decreased to as little as 25µl. Do not use less than 25µl of Elution Buffer.



Notes:

1. Ensure that the Elution Buffer is in the bottom of the tube. If Elution Buffer is on the side of the tube, the elution may be suboptimal.
2. Use only the Elution Tubes provided with the kit; other tubes may not work with the Maxwell® 16 Instrument.

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

Note: The total volume of the sample should not exceed 100µl. Adding more than 100µl of cell suspension may result in poor yields.

5. Proceed to Section 5.B for Cat.# AS3000 instruments. Proceed to 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.

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 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 Elution Buffer 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:

- 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.

16. 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 more detailed information.

To run this protocol, you must have Maxwell® 16 firmware version 4.5 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.

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

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 Elution Buffer 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:

- 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.

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: • Process a larger number of cells (up to 10^4 cells) to increase yield. • Take care not to disturb the cell pellet when removing the sample supernatant.
Poor PCR results	Too much starting material. Reduce the amount of sample used for purification.
	Wrong elution buffer was added. Use only the Elution Buffer supplied with the Maxwell® 16 Cell LEV DNA Purification Kit.
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.
	Incorrect elution tube used with the system. To prevent a Z-axis collision, only use the 0.5ml elution tube provided with the Maxwell® 16 Cell LEV DNA Purification Kit. Other tubes may have different dimensions.
Resin carryover during elution	A small amount of resin is visible in elution tube. The presence of resin particles in the elution tube will not affect the final DNA concentration or downstream applications. If desired, an additional resin capture step may be performed using the 0.5ml MagneSphere® Stand (Cat.# Z5341) or Maxwell® 16 LEV Elution Magnet (Cat.# AS1261).

7. Related Products

Instrument and Hardware Accessories

Product	Cat.#
Maxwell® 16 Instrument	AS2000
Maxwell® 16 MDx Instrument	AS3000
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 Viral Total Nucleic Acid Purification Kit	48 preps	AS1150
Maxwell® 16 LEV Blood DNA Kit	48 preps	AS1290

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

[a]U.S. Pat. Nos. 6,027,945 and 6,368,800, Japanese Pat. No. 3253638 and other patents pending.

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

[c]Patent Pending.

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