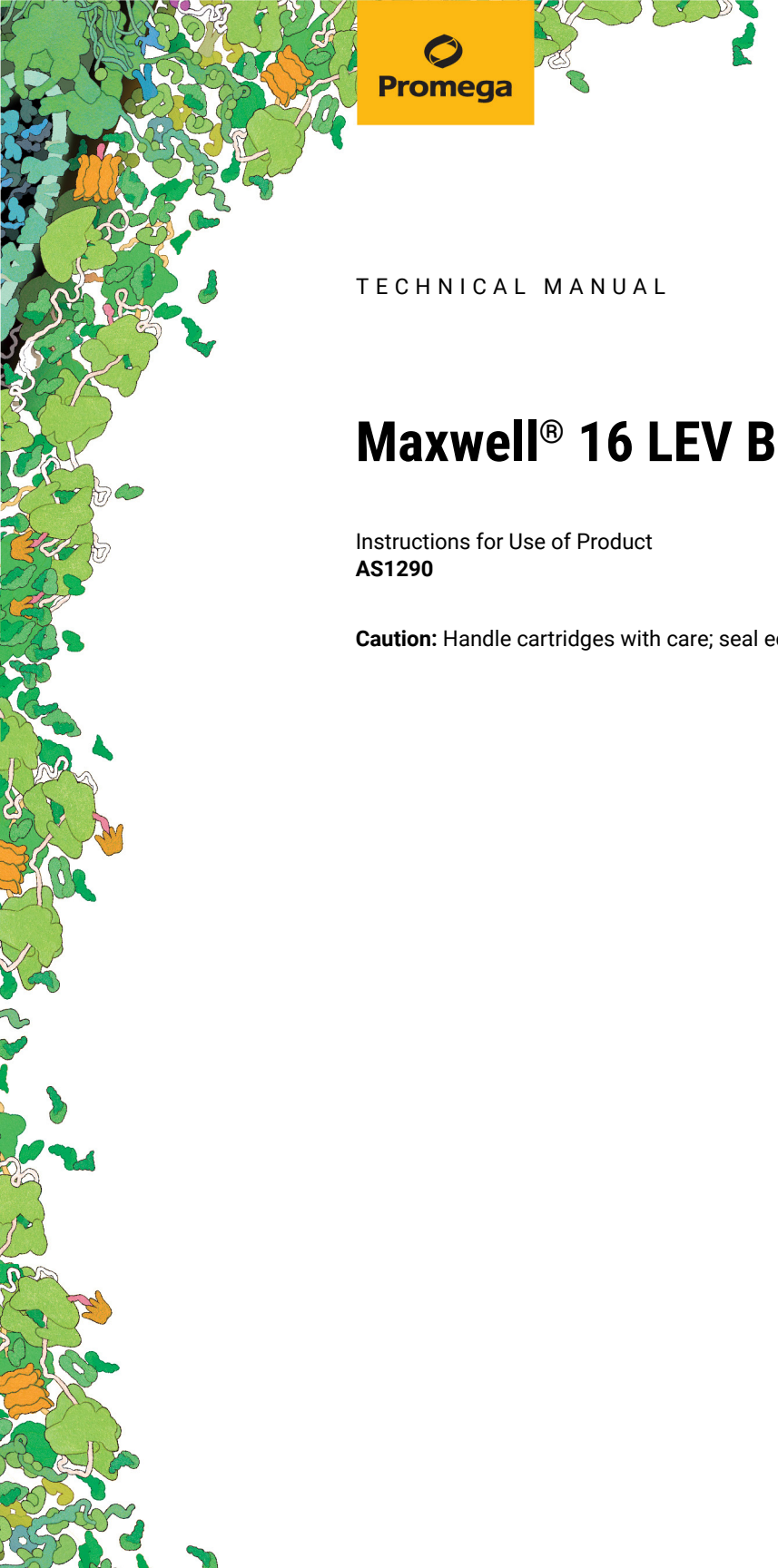

TECHNICAL MANUAL

Maxwell® 16 LEV Blood DNA Kit

Instructions for Use of Product
AS1290

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

Revised 2/25
TM333

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Maxwell® 16 LEV Blood DNA Kit

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 LEV Blood DNA Kit is used with the Maxwell® 16 Instrument to provide an easy method for efficient, automated purification that concentrates genomic DNA (gDNA) from whole blood samples. The Maxwell® 16 Instrument is 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 40 minutes. The purified DNA can be used directly in a variety of downstream applications, including PCR and agarose gel electrophoresis.

The Maxwell® 16 Instrument purifies samples using a novel paramagnetic particle, called the MagnaCel™ particle, which provides a mobile solid phase that optimizes sample capture, washing and purification of gDNA. This particle utilizes cellulose-based binding of nucleic acids and provides a higher bind capacity and cleaner eluate than traditional silica-based DNA purification. The Maxwell® 16 Instrument is a magnetic particle-handling instrument that efficiently binds gDNA to the paramagnetic particle in the first well of a prefilled cartridge and mixes during processing. The approach to magnetic capture 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

PRODUCT	SIZE	CAT.#
Maxwell® 16 LEV Blood DNA Kit	**48 preps**	**AS1290**

For Laboratory Use. Sufficient for 48 automated isolations from 300µl of whole blood samples. Includes:

- 2 × 1ml Proteinase K (PK) Solution
- 20ml Lysis Buffer
- 48 Maxwell® 16 LEV Cartridges (MCD)
- 50 LEV Plungers
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® 16 LEV Blood DNA Kit at +15°C to +30°C.

Available Separately (recommended for sample extraction)

PRODUCT	SIZE	CAT.#
ClickFit Microtube, 1.5ml	**1,000/pack**	**V4741**

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

The Maxwell® 16 reagent cartridges are designed to be used with potentially infectious substances. Users should wear 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.

3. Before You Begin

Maxwell® 16 Instrument Hardware and Firmware Setup

To use the Maxwell® 16 LEV Blood DNA 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. Refer to the *Maxwell® 16 Instrument Technical Manual* specific for your instrument for directions.

Materials to Be Supplied by the User

- **optional:** rotating tube mixer for liquid blood samples
- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for incubation of samples (e.g., ClickFit Microtube, 1.5ml [Cat.# V4741]; recommended to prevent the cap from opening during heating)
- heating block set at 56°C

3.A. Preparation of Whole Blood Samples

Whole Blood Sample Processing Capacity

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 LEV Blood DNA Purification Kit is designed to purify genomic DNA from up to 300µl of whole blood, assuming an average number of white blood cells in the range of 4×10^6 to 1.1×10^7/ml whole blood (values for a normal healthy adult; 1).

Note: Whole blood samples collected in EDTA, ACD or heparin tubes can be used. These samples may be either fresh or frozen. Frozen samples should be thawed before processing. We recommend mixing all blood samples before use. EDTA blood collection tubes are preferred if the purified DNA will be used in downstream amplification assays.

1. Mix all blood samples for at least 5 minutes at room temperature.
2. Prepare and label incubation tubes compatible with heating block.
3. Add 30µl of Proteinase K (PK) Solution to each incubation tube.
4. Add liquid blood (up to 300µl) to each incubation tube.
5. Add 300µl of Lysis Buffer to each incubation tube.
6. Vortex each tube for 10 seconds.
7. Incubate each tube in the heating block (set to 56°C) for 20 minutes. During this incubation, prepare cartridges as described in Section 3.B.
8. Transfer each blood lysate sample from the incubation tube to well #1 of each cartridge. (Well #1 is the well closest to the cartridge label and furthest from the user.)

3.B. Maxwell® 16 Cartridge Preparation

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.
2. Place one plunger into well #8 of each cartridge.
3. Place an empty Elution Tube into the Elution Tube position for each cartridge in the Maxwell® 16 LEV Cartridge Rack. Add 50µl of Elution Buffer to the bottom of each Elution Tube.

Notes:

a. If you are processing fewer than 16 samples, center the cartridges on the platform.

b. Specimen or reagent spills on any part of the Maxwell® 16 LEV Cartridge Rack should be cleaned with a detergent-water solution, followed by a bacteriocidal spray or wipe, then water. Do not use bleach on any instrument parts.

Well	Contents	User Adds:
1.	Binding Buffer	Sample
2.	Paramagnetic Cellulose Particles	
3.	Wash Buffer	
4.	Wash Buffer	
5.	Wash	
6.	Wash	
7.	Water	
8.	Empty	Plunger

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Figure 1. Maxwell® 16 LEV DNA Purification Cartridge. This figure shows the contents of a cartridge. In all cases, lysate sample is added to well #1.

Figure 2. Setup and configuration in the Maxwell® 16 LEV Cartridge Rack. Elution Buffer is added to the Elution Tubes as indicated.

4. Instrument Run: AS2000 and AS3000 Instruments

4.A. Setup for AS2000 Maxwell® 16 Instruments

Refer to the *Maxwell® 16 Instrument Operating Manual* #TM295 for more detailed information.

To run the "Blood" 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, then select **OK** at the Verification screen.
5. Select **Blood** on the Menu screen, then select **OK** at the Verification 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 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:

    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 #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.

Note: Following the automated purification procedure, the LEV Cartridge Rack will be warm. It will not be too hot to touch. To remove the rack from the instrument platform, hold onto the sides of the rack.

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.

4.B. Setup for AS3000 Maxwell® 16 MDx Instruments

Refer to the *Maxwell® 16 MDx Instrument Technical Manual* #TM320 for detailed information. To run the "Blood" protocol, you must have the Maxwell® 16 Firmware version 1.10 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. At the Protocols screen, select **DNA**.
5. At the Method screen, select **Blood**.
6. On the next screen, verify that the correct user was chosen. The protocol should read "DNA". 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.

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4.B. Setup for AS3000 Maxwell® 16 MDx Instruments (continued)

10. Verify that samples were added to well #1 of the cartridges, cartridges 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 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 #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 cap the tubes.

Note: Following the automated purification procedure, the LEV Cartridge Rack will be warm. It will not be too hot to touch. To remove the rack from the instrument platform, hold onto the sides of the rack.

16. 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.

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

5. Reference

1. Henry, J.B. (2001) *Clinical Diagnosis and Management by Laboratory Methods*, 20th ed., W.B. Saunders Company, 509.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptoms	Causes and Comments
Lower than expected A_{260} (lower than expected yield)	Blood that has undergone multiple freeze-thaw cycles may have degraded DNA. Use fresh samples whenever possible, or avoid multiple freeze-thaw cycles.
	Proteinase K Solution was not added. The lysis and yield are dependent upon complete extraction with proteinase K. If proteinase K was not added in Section 3.A, Step 3, the resulting blood sample will be red. Proteinase K-treated samples turn greenish brown. This can be used as a quick diagnostic method of determining whether or not the proteinase K was added.
	Whole blood sample contained 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.
	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.

7. Related Products

Product	Size	Cat.#
Maxwell® 16 LEV Hardware Kit	1 each	AS1250
RNase A Solution, 4mg/ml	1ml	A7973
ClickFit Microtube, 1.5ml	1,000/pack	V4741

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8. Summary of Changes

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

1. Removed discontinued product Maxwell® 16 Buccal Swab LEV DNA Purification Kit (Cat.# AS1295).
2. Deleted original Section 3.B and renumbered Section 3.C as Section 3.B.
3. Added Section 4.A and 4.B to AS2000 and AS3000 instrument setups, respectively.
4. Updated the cover page and document font.
5. Made minor text edits.