TECHNICAL MANUAL

# Maxwell® 16 LEV Plant DNA Kit

Instructions for Use of Product **AS1420**

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


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TM414

# Maxwell®16 LEV Plant DNA 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



## 1. Description

The Maxwell® 16 LEV Plant DNA Kit is used with the Maxwell® 16 Instrument (AS2000) to provide an easy method for efficient, automated purification of genomic DNA (gDNA) from plant tissue samples. The Maxwell® 16 Instrument is supplied with preprogrammed purification methods and is designed for use with predispensed reagent cartridges, maximizing simplicity and convenience. The instrument can process up to 16 samples in 45 minutes and the entire procedure can be completed in approximately 60 minutes, depending on the preprocessing method chosen. 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 cellulose-based particle provides a higher binding capacity and higher concentration eluates than silica-based DNA purification methods. The Maxwell® 16 Instrument is a magnetic particle handler that efficiently binds gDNA to the paramagnetic particles in the first well of the reagent cartridge and mixes during processing. This approach to magnetic capture avoids common problems such as clogged tips or partial reagent transfers that result in suboptimal purification by other commonly used automated systems.

## 2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **Maxwell® 16 LEV Plant DNA Kit** | **48 preps** | **AS1420** |

Sufficient for 48 automated isolations from plant lysate samples. Includes:

- 25ml Tail Lysis Buffer (TLA)
- 25ml Nuclease-Free Water
- 1ml RNase A (4mg/ml)
- 48 Maxwell® 16 LEV Cartridges (MCD)
- 50 LEV Plungers
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

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

**Safety Information:** The reagent cartridges contain ethanol and isopropanol. These substances should be considered flammable, harmful and irritants. Wear gloves and goggles when handling.

**Available Separately** ClickFit Microtubes (Recommended for use with the liquid nitrogen sample extraction method; See Section 3.C.)

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

## 3. Before You Begin

### Maxwell® 16 Instrument Hardware and Firmware Setup

To use the Maxwell® 16 LEV Plant 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. Refer to the Maxwell® 16 Instrument (AS2000) Operating Manual #TM295 for directions.

### 3.A. Maxwell® 16 Cartridge Preparation

Catridges should be prepared **immediately before Step 7** in Section 3B or **Step 8** in Section 3C.

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:**

   1. If you are processing fewer than 16 samples, center the cartridges on the platform.
   2. Sample 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, the sample lysate is added to well #1.

### 3.A. Maxwell® 16 Cartridge Preparation (continued)


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

### 3.B. Preparation of Plant Leaf Samples with a Mechanical Bead-Beating Device

This preprocessing protocol requires a mechanical bead-beating device with a bead and tube, or bead and sealable deep-well plate, combination.

**Materials to Be Supplied by the User**

- Bead-beating device (e.g., MP Biomedicals FastPrep®-24 Instrument)
- Sterile, aerosol-resistant pipette tips for sample transfer into prefilled reagent cartridges
- Microcentrifuge or plate-specific centrifuge

**Sample Processing Notes**

The total yield of genomic DNA from plant materials depends on the volume of material processed and the amount of genomic DNA in the plant material used. Each cartridge supplied in the Maxwell® 16 LEV Plant DNA Kit is designed to purify genomic DNA from 310µl of plant lysate. The materials required to generate the plant lysate are also supplied in the kit. Nuclease-Free Water is provided to dilute the binding buffer in the first well of the cartridge to optimize binding of the genomic DNA.

1. Place up to 20mg leaf tissue in the bottom of each tube or well.
2. Place a bead (or beads, as recommended by manufacturer) into each tube or well.
3. Add 300µl of Tail Lysis Buffer (TLA) to each tube or well.
4. Add 10µl of RNase A (optional, to eliminate RNA) to each well.

   **Note:** If you are processing a large number of samples, prepare sufficient volume of Tail Lysis Buffer and RNase A immediately before use and add 310µl of this cocktail to each sample.
5. Run the bead beating device using the time and speed recommended by the manufacturer. Some optimization may be required to generate sufficient sample lysis for the desired DNA yield.

6. Place the extraction tubes or plates into a centrifuge and spin briefly to remove any solid particulates from the sample lysate. **Optional:** To reduce foaming of the solution, centrifuge for up to 2 minutes at maximum speed.
7. Add 300µl of Nuclease Free Water to well #1 of each Maxwell® 16 LEV Plant DNA Kit reagent cartridge. (Well #1 is the well closest to the cartridge label and furthest from the user).
8. Transfer each plant lysate sample from the extraction tube or plate into well #1 of a reagent cartridge. Transfer all liquid and any remaining foam, being careful not to transfer any solid material to the cartridge.

### 3.C. Preparation of Plant Leaf Samples with a Microtube, Pestle and Liquid Nitrogen

This preprocessing protocol uses a mortar and pestle for tissue grinding, and liquid nitrogen to freeze the sample.

**Materials to Be Supplied by the User**

- Pellet Pestles (Sigma Aldrich Cat.# Z359947)
- Liquid nitrogen
- Sterile, aerosol-resistant pipette tips for sample transfer into prefilled reagent cartridges
- Microcentrifuge

**Sample Processing Notes**

The total yield of genomic DNA from plant materials depends on the volume of material processed and the amount of genomic DNA in the plant material used. Each cartridge supplied in the Maxwell® 16 LEV Plant DNA Kit is designed to purify genomic DNA from 310µl of plant lysate. The reagents required to generate the plant lysate are also supplied in the kit. Nuclease-Free Water is provided to dilute the binding buffer in the first well of the cartridge to optimize binding of the genomic DNA.

1. Place up to 20mg leaf tissue in the bottom of a ClickFit Microtube, 1.5ml.
2. Add liquid nitrogen to the plant tissue sample. Allow the liquid to evaporate, freezing the sample.
3. Using a pellet pestle, grind the frozen plant tissue against the tube wall as thoroughly as possible.
4. Add 300µl of Tail Lysis Buffer (TLA) to each tube.
5. Add 10µl of RNase A (optional, to eliminate RNA) to each tube.

   **Note:** If you are processing a large number of samples, combine sufficient volume of Tail Lysis Buffer and RNase A immediately before use and add 310µl of this cocktail to each sample.
6. Vortex tube briefly (10 seconds).
7. Place tubes with lysate into a microcentrifuge and spin briefly to remove solid particulates from the lysate. **Optional:** To reduce foaming of the solution, centrifuge for up to 2 minutes at maximum speed.
8. Add 300µl of Nuclease Free Water to well #1 of each Maxwell® 16 LEV Plant DNA Kit reagent cartridge. (Well #1 is the well closest to the cartridge label and furthest from the user).
9. Transfer each plant lysate sample from the extraction tube to well #1 of the reagent cartridge. Transfer all liquid and any remaining foam, being careful not to transfer any solid material to the cartridge.

## 4. Instrument Run: AS2000 Instrument

### Setup for Maxwell® 16 Instrument (AS2000)

Refer to the *Maxwell® 16 Instrument (AS2000) Operating Manual* #TM295 for more detailed information.

To run the "Plant" protocol, you must have Maxwell® 16 firmware version 4.97 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 is set at "LEV" hardware configuration and "Rsch" operational mode.
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 "Plant" 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 onto 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:**

    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 the on-screen instructions at the end of the method to open the 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.

## 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 |
|---|---|
| Lower than expected $A_{260}$ (yield) | Insufficient lysis. Consider optimization of the extraction protocol. If using a mechanical bead-beating device, consider increasing the number of strokes/minute or the amount of processing time. |
| | Sample is relatively poor in DNA content. Use more starting material. |
| | Solid material in cartridge. Transferred lysate may contain too much solid material, which may interfere with particle handling on the Maxwell® 16 instrument. |
| Resin fines are present in the eluate | Briefly centrifuge and transfer the eluate to a clean tube. |
| Lower than expected absorbance ($A_{260}$:$A_{280}$ or $A_{260}$:$A_{230}$) ratio | The MagnaCel™ particles may co-isolate plant compounds that can affect the absorbance ratio. Use an amplification-based assay to assess the quality and suitability of the isolated DNA for downstream PCR analysis. |

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