TECHNICAL BULLETIN

Wizard® Magnetic 96 DNA Plant System

Instructions for Use of Products
FF3760 and FF3761

Revised 5/17
TB289

Wizard® Magnetic 96 DNA Plant System

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

Isolation of genomic DNA from agricultural crop materials using traditional procedures can be a lengthy and potentially hazardous process. Further, many procedures may have substantial carryover of inhibitors, limiting their usefulness. The Wizard® Magnetic 96 DNA Plant System[a] eliminates lengthy incubations and the use of hazardous organic solvents such as chloroform and phenol while gaining the ability to perform plant genomic DNA purification procedures in a 96-well plate format. These procedures can be performed manually or automated on a Beckman Coulter Biomek® 2000 workstation.

The Wizard® Magnetic 96 DNA Plant System purifies DNA from commercially important agricultural crops such as corn and tomato leaf as well as canola and sunflower seeds. The DNA purified from these samples can be used in PCR as well as more stringent applications such as RAPD analysis.

The Wizard® Magnetic 96 DNA Plant System uses MagneSil® Paramagnetic Particles (PMPs). Paramagnetic particles can be considered a “mobile solid phase”. Unlike column-based systems, binding of nucleic acids to magnetic particles can occur in solution, resulting in increased binding kinetics and binding efficiency. Particles also can be completely resuspended during the wash steps of a purification protocol, thus enhancing the contact with, and removal of, contaminants, increasing nucleic acid purity.

1. Description (continued)

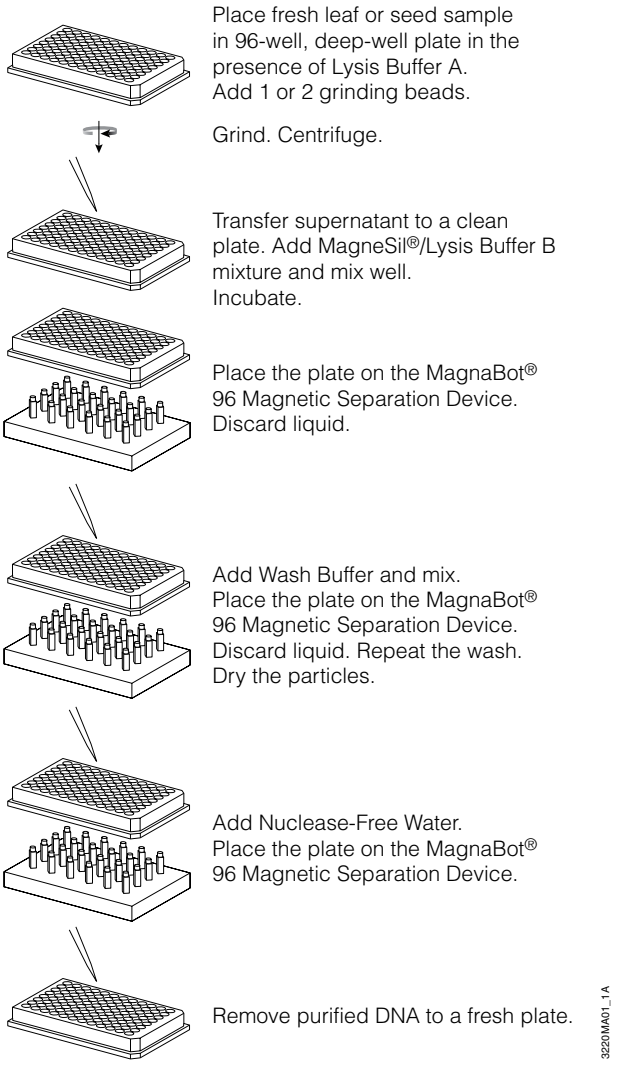

Figure 1. Schematic of DNA isolation using the Wizard® Magnetic 96 DNA Plant System.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Wizard® Magnetic 96 DNA Plant System	**2 × 96 preps**	**FF3760**

Each system contains sufficient reagents to perform approximately 2 × 96-well plate preparations. The Wizard® Magnetic 96 DNA Plant System includes:

- 68ml Lysis Buffer A, Plant
- 14ml Lysis Buffer B, Plant
- 2.25ml MagneSil® Paramagnetic Particles
- 40ml Wash Buffer, Plant
- 4 U-bottom 96-well plates

PRODUCT	SIZE	CAT.#
Wizard® Magnetic 96 DNA Plant System	**4 × 96 preps**	**FF3761**

Each system contains sufficient reagents to perform approximately 4 × 96-well plate preparations. The Wizard® Magnetic 96 DNA Plant System includes:

2 × Cat.# FF3760:

- 136ml Lysis Buffer A, Plant
- 28ml Lysis Buffer B, Plant
- 4.5ml MagneSil® Paramagnetic Particles
- 80ml Wash Buffer, Plant
- 8 U-bottom 96-well plates

Storage Conditions: Store all components at room temperature (22–25°C).

Do not freeze the MagneSil® Paramagnetic Particles.

3. Protocols

3.A. Isolation of DNA from Leaf Tissue and Seeds in a 96-Well Plate

Materials to Be Supplied by the User

- MagnaBot® 96 Magnetic Separation Device (Cat.# V8151)
- MagnaBot® Spacer (Cat.# V8381)
- TE (pH 8.0) or Nuclease-Free Water
- 8-channel pipette (50–200µl)

Grinders

Geno/Grinder® 2000 (SPEX CertiPrep, Inc.)

- 96-well, deep-well plates, 1 or 2ml (polypropylene)
- sealing tape (3M Scotch® brand aluminum foil tape 425: 3 inches × 60 yards)
- Geno/Grinder® beads

OR

Retsch MM300 Mixer Mill, plate holder and consumables

Before You Begin

Prepare ethanol wash solution by adding 20ml of 96–100% ethanol and 20ml of isopropanol (IPA) to the Wash Solution bottle, and mix well. After alcohol addition, the total volume of the Wash Buffer, Plant, should be 80ml. Store any unused wash solution sealed tightly at room temperature.

Prepare sufficient MagneSil®/Lysis Buffer B solution for the number of samples to be processed. **Prepare fresh just prior to use.**

For eight samples (one column), add 85µl of resuspended MagneSil® PMPs to 540µl of Lysis Buffer B. Only 480µl of MagneSil®/Lysis Buffer B solution is added per column of the 96-well plate. For more samples, increase volumes proportionally.

Protocol

1. Place 8mm fresh leaf disks or seed samples (1–5) in a sealed 96-well, deep-well plate (Geno/Grinder®) or capped microtubules (Retsch) in the presence of 300µl of Lysis Buffer A and 1 or 2 grinding beads.
2. Process in the Geno/Grinder® or MM300 Mixer Mill following the manufacturer's instructions. **Times and speeds will need to be determined for each sample type.**

   Note: Leaf samples are more easily homogenized, and seeds may need longer times and/or higher speeds. Use the minimum speed that produces an even suspension to prevent excess shearing of the DNA.
3. Centrifuge 96-well deep-well plates at 1,700 × *g* for 10 minutes to collect cell debris.
4. Transfer 125µl of each sample to the appropriate well of the provided U-bottom plate.

   Note: Avoid aspirating the pellet from the bottom of the wells. If there is a layer of debris or oil on the surface of the liquid, place the pipette tip under it to avoid carryover to the new plate.

5. Add 60µl/well of MagneSil®/Lysis Buffer B mixture, and pipet to mix well.
6. Incubate at room temperature for 5 minutes, mixing once by pipetting. Use fresh tips to avoid cross-contamination.
7. Place the plate onto the MagnaBot® 96 Magnetic Separation Device with MagnaBot® Spacer for 1 minute. Discard the liquid by pipetting/aspiration.
8. Remove the plate from the MagnaBot® 96 Magnetic Separation Device, and add 150µl of wash solution. Resuspend the MagneSil® PMPs by mixing for 10–15 seconds.
9. Place the plate on the MagnaBot® 96 Magnetic Separation Device with MagnaBot® Spacer for 30 seconds, and remove the liquid as in Step 7.
10. Repeat the wash once more using 100µl of wash solution per well.
11. After the last wash, remove as much liquid as possible and dry the particles at room temperature for 5 minutes.
12. Remove the plate from the MagnaBot® 96 Magnetic Separation Device, and add 50µl of TE buffer or nuclease-free water. Thoroughly resuspend the MagneSil® PMPs, and incubate at room temperature for 5 minutes.
13. Place the plate on the MagnaBot® 96 Magnetic Separation Device with MagnaBot® Spacer for 1 minute. Transfer the purified DNA to a fresh U-bottom plate.

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3.B. Isolation of DNA from Leaf Tissue and Seeds Using the Biomek® 2000 Workstation

This protocol uses the Beckman Coulter Biomek® 2000 workstation to automate DNA isolation from plant tissue and seeds. For more information, refer to the documentation provided with BioWorks™ methods. BioWorks™ methods are available for download at: **www.promega.com/applications/automat/**

Materials to Be Supplied by the User

- Biomek® 2000 workstation (Beckman Coulter, Inc.)
- MagnaBot® 96 Magnetic Separation Device (Cat.# V8151)
- MagnaBot® Spacer (Cat.# V8381; placed on top of MagnaBot® 96 Magnetic Separation Device during purification)
- MP200 tool
- tool rack
- 2 × 1/4 vertical reagent holders
- 2 × 1/4 single reagent holder
- 96-square-well, deep-well plate
- 2 × P250 tip rack assembly (nonsterile)

Grinders

Geno/Grinder® 2000 (SPEX CertiPrep, Inc.)

- 96-well, deep-well plates, 1 or 2ml (polypropylene)
- sealing tape (3M Scotch® brand aluminum foil tape 425: 3 inches × 60 yards)
- Geno/Grinder® beads

OR

Retsch MM300 Mixer Mill, plate holder and consumables

Before You Begin

Prepare ethanol wash solution by adding 20ml of 96–100% ethanol and 20ml of isopropanol to the Wash Solution bottle, and mix well. After alcohol addition, the total volume of the Wash Buffer, Plant, should be 80ml. Store any unused wash solution sealed tightly at room temperature.

Prepare sufficient MagneSil®/Lysis Buffer B solution for the number of samples to be processed. **Prepare fresh just prior to use.**

Do not store the MagneSil®/Lysis Buffer B mixture.

For each 96-well plate, add 1.1ml of thoroughly resuspended MagneSil® PMPs to 7.0ml of Lysis Buffer B. Only 5.7ml of MagneSil®/Lysis Buffer B is added per plate. This extra volume accounts for the needed overage when using an automated liquid handler. Place the mixture in a reagent holder, and mix well before each addition. Discard excess after the run is complete.

Protocol

1. Place 8mm fresh leaf disks or seed samples (e.g., 1–5 canola seeds) in a sealed 96-well, deep-well plate (Geno/Grinder®) or capped microtubules (Retsch) in the presence of 300µl of Lysis Buffer A and 1 or 2 grinding beads.
2. Process in the Geno/Grinder® or MM300 Mixer Mill following the manufacturer's instructions. Times and speeds will need to be determined for each sample type.

   Note: Leaf samples are more easily homogenized, and seeds may need longer times and/or higher speeds. Use the minimum speed that produces an even suspension to prevent excess shearing of the DNA.
3. Centrifuge 96-well, deep-well plates at 1,700 × *g* for 10 minutes to spin down cell debris.
4. Place the plate or microtube rack on the Biomek® 2000 and begin running the appropriate BioWorks™ method.

   Note: BioWorks™ methods are available for download at: **www.promega.com/applications/automat/**

The robotic protocol is programmed to transfer 125µl of cleared lysate from the Retsch collection microtube plate without disturbing the pelleted debris. This debris can interfere with subsequent purification. It is very important that the recommended ratio of seeds or leaf tissue to lysis buffer not be exceeded, as this will cause the tips to disturb the pellet and subsequent transfer to the purification plate. In addition, if a different extraction plate or method is used, it will be necessary to re-optimize the tip height before performing the transfer step.

The BioWorks™ methods available from Promega is optimized for the purification steps of this system. This includes the speeds and locations of all aspiration and dispensing steps. If a different robotic workstation is used, these parameters will have to be adjusted for the new robot. The speed of the aspiration during the wash steps must be slow enough so that the MagneSil® PMPs are not sheared from the sides of the wells and discarded in the wash solution. Similarly, the speed of dispensing must be fast enough so that the particles are resuspended and slow enough so that no aerosols are produced and no splashing occurs.

The last step of the purification process involves aspirating the last wash, drying, then resuspending the particles in TE buffer or nuclease-free water to release the purified DNA. The Biomek® 2000 protocol is designed to remove all traces of the ethanol wash solution from the supplied 96-well plate.

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4. Related Products

Product	Size	Cat.#
MagnaBot® 96 Magnetic Separation Device	1 each	V8151
MagnaBot® Spacer	1 each	V8381
Nuclease-Free Water	50ml (2 × 25ml)	P1193
TE Buffer, 1X, Molecular Grade	100ml	V6231
	500ml	V6232

5. Summary of Changes

The following changes were made to the 5/17 revision of this document:

1. Discontinued products were removed from Related Products.
2. The document design was updated.

[a]U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756, Japanese Pat. No. 3253638, Mexican Pat. No. 209436 and other patents pending.

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