AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® Plant DNA Plus Kit

Version 2.1

Magnetic bead-based kit for isolation of genomic DNA from plant samples

Catalog Number: D018-00, D018-01, D018-02,

Contents

Disclaimers and Safety Information

This kit is designed for research use only. All biological samples are considered potentially infectious. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Documents" tab when viewing the product kit. Download MSDS at www.amdbiotech.com. Information in this document is subject to change without notice.

Product Introduction

Auto-Mag® Plant DNA Plus Kit uses Auto-Mag® magnetic beads technology and is specially designed for rapid and reliable purification of gDNA from a wide range of plant and fungi species. The kit also includes additional reagents and protocol for the purification of plant pathogen DNA along with the purification of the total plant DNA. Purified DNA is suitable for various conventional operations, including real time PCR, Southern blotting, SNP analysis, Enzyme digestion, Next Generation Sequencing, chip detection, hybridization applications and other experiments. The protocols can be used for manual procedures as well as guidelines for adapting it to magnetic rod automatic nucleic acid purification instruments.

Features

- Isolate high quality total DNA from a variety of plant and fungal species, including pathogen DNA
- Highly specific binding of DNA allows easy removal of impurities and inhibitors.
- Work for different plant species and tissues
- Eluted DNA is suitable for most downstream applications.
- Automation-friendly

Kit Contents

Product Number	D018-00	D018-01	D018-02
Preparation	5	96	384
Auto-Mag® D-3	0.06 ml	1.1.ml	4.4 ml
PDL Buffer	4 ml	60 ml	240 ml
PLE 1 Buffer	0.4 ml	6 ml	24 ml
PDB Buffer	4 ml	50 ml	200 ml
MDW Buffer*	4 ml	60 ml	120 ml x2
DNA Elution Buffer	1 ml	15 ml	60 ml
LEM-1	0.15 ml	3 ml	12 ml
IHR reagent	1.5 ml	25 ml	100 ml
RNase A	-	0.6 ml	2.4 ml
* Ethanol must be added prior to use. See Preparation of Reagents			

Storage and Stability

Auto-Mag® Plant DNA Plus Kit is shipped at ambient temperature. All components are stable for 12 months when stored accordingly. LEM-1 (lysis enzyme mix-1), and IHR (inhibitor remove reagent) should be stored at 2-8°C. Auto-Mag® D-3, and RNase A can be stored at room temperature (15-25°C) for 12 months, to prolong the shelf-life, storage at 2-8°C is recommended. PDL Buffer with RNase A mix should be stored at 2-8°C. All other components can be stored at room temperature (15-25°C).

During shipment or storage in cool ambient conditions, the precipitates may form in some buffers. Check buffers and re-dissolve any precipitates by warming the buffer at 37˚C. and gently shaking before using.

Preparation of Reagents

1. Add RNase A to the bottle of PDL before use. Store at 2-8°C

Reagents	Kit	RNase A to be Added
PDL Buffer	D018-00	*
	D018-01	0.6 ml
	D018-02	2.4 ml
*RNase A has already been added to PDL buffer prior to shipping out.		

2. Dilute MDW Buffer with 100% Ethanol as follows and store at room temperature.

Reagents	Kit	100% Ethanol to be Added
MDW Buffer	D018-00	4 ml
	D018-01	60 ml
	D018-02	120 ml per bottle
Components are stable for one year when stored closed at room temperature		

3. Freshly prepared 70% ethanol for DNA wash steps and prepare at least 1.2 ml per sample preparation.

Additional Information

1. Specification

Features	Specification
Isolation Technology	Magnetic Beads
Sample Sources	Plant tissue, Seed, etc.
Starting Amount	Up to 50mg
Typical Yield	Dependent upon sample
Elution Volume	50-100 µl
Processing format	Auto, Manual
Downstream Application	NGS, PCR, qPCR, real-time RT-PCR, and Sequencing etc.

Auto-Mag® Plant DNA Isolation Protocols

Materials and Equipment to Be Supplied by User

- 2.0 ml Homogenizer Bead Beating Tubes, or Prefilled Tissue Disruptor Tube (RNase free), (Cat# AMD B014)
- Equipment for disrupting plant tissue (Geno/Grinder, Mixer Mill, or Bead Mill Homogenizers etc.)
- 3-5 mm tungsten carbide beads
- Single-tube format: Nuclease-free 1.5 ml microcentrifuge tube
- 96-well format: 2 ml deep-well plate
- Sealing film
- Magnetic separation device
- Vortex
- Centrifuge capable of ≥16,000 × g for microcentrifuge tubes
- Centrifuge capable of 3000–4000 × g with swinging-bucket rotor for 96-well deep well plates
- Water bath, incubator, or heat block capable of 40°C, 65°C, and 70°C
- Liquid nitrogen for freezing/disrupting samples
- 70% Ethanol
- 100% Ethanol
- 100% Isopropanol

Before Starting

- Please to read this protocol in its entirety to become familiar with the procedures
- Prepare reagents according to instructions of Preparation Reagents on page 3
- Prepare 70% ethanol for DNA wash steps. Do not use a previously prepared solution.
- Re-dissolve any precipitates of PLE-1 by warming to 37˚C.
- Preset water bath, incubator, or heating blocks to 40°C, 65°C, and 70°C
- Complete resuspension of the Auto-Mag® D-3 beads by vortex

Protocol for Single-tube manual format

1. Disrupt the plant tissue：

(1) Disrupt the sample mechanically

Plant tissue can be homogenized with a rotor-stator, bead mill, or high-throughput homogenizers. We recommend bead mill or high-throughput homogenizers paired with stainless steel beads. When using beads for homogenization, ensure that the correct tubes are used, which typically have thicker walls and a screw cap with an O-ring.

- Place 30-50mg of fresh plant tissue or 10–20 mg of seed samples into 2.0 ml Homogenizer Bead Beating Tubes in the presence of one or two grinding beads.
- Grind sample using a mechanical grinder following the manufacturer's instructions.
- After sample homogenization, go to step 2

(2) Disrupt the sample manually:

- Place 30-50mg of fresh plant tissue or 10–20 mg of seed samples into a mortar that contains liquid nitrogen,
- Grind the tissue thoroughly using a clean pestle, then allow the liquid nitrogen to evaporate
- Transfer the powder to a Nuclease-free 1.5 ml microcentrifuge tube and go to step 2

2. Add 500µl PDL Buffer and 50µl of PLE-1, vortex at maximum speed for 20 seconds or pipette mix 20 times.

Note: If there are clumps of plant matter on the top of the sample tube, please pulse centrifugation to ensure the plant matter is covered by the PDL Buffer.

3. Incubate the sample at 70ºC for 30 minutes. Occasionally mix the sample 1-2 times during incubation.

4. Cool sample at room temperature for 5 minutes.

5. Add 200µl of IHR reagent and vortex at maximum speed for 20 seconds. Incubate samples at room temperature for 2 minutes.

Note: Completely resuspend IHR Reagent by shaking or vortexing the bottle before use. If necessary, use wide-bore tips (or cut the tips to form a wider tip) for the ease of pipetting of the IHR Reagent.

6. Centrifuge the sample tube at 16,000g x g for 10 min. Collect and transfer ~400µl cleared lysate to a new tube.

Note: Use centrifuge for microcentrifuge tubes. Do not transfer any pellet or other debris.

7. Add 400µl PDB Buffer, 200µl 100% isopropanol and 10µl Auto-Mag® D-3, vortex at maximum speed for 20 seconds or pipette mix 20 times. Incubate the sample tube at room temperature for 8 minutes.

Note: PDB Buffer, isopropanol and Auto-Mag® D-3 can be prepared as a master mix prior to use. Prepare only what is needed and mix completely. Complete resuspension of the Auto-Mag® D-3 beads are crucial for obtaining purity.

8. Place the samples tube on a compatible magnetic separation device for 3 minutes or until Auto-Mag® D-3 beads are completely cleared from the solution. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample tube from the magnet. Add 600µl MDW Buffer and resuspend the Auto-Mag® D-3 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times.

   Note: MDW Buffer must be diluted with ethanol prior to use. Complete resuspension of the magnetic beads is critical for obtaining good purity DNA.

10. Place the sample tube on the magnet for 3 minutes or until Auto-Mag® D-3 beads are completely cleared from the solution. Remove and discard all the liquid. Do not disturb the attracted beads.

11. Repeat Steps 9-10 for second MDW wash.

12. Remove the sample tube from the magnet. Add 600µl of freshly prepared 70% ethanol and resuspend the Auto-Mag® D-3 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times.

13. Place the sample tube on the magnet for 5 minutes or until Auto-Mag® D-3 beads are completely cleared from the solution. Remove and discard all the liquid. Do not disturb the attracted beads.

14. Repeat Steps 10-11 for second 70% ethanol wash.

15. Keep the sample tube on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

   Note: *It is critical to completely remove all liquid from each tube.*

16. Remove the tube off the magnetic separation device, add 50-100µl Elution Buffer to the sample and resuspend the Auto-Mag® D-3 beads by vortex for 20 seconds or pipette mix 20 times. Incubate the sample tube at 65°C for 5 minutes.

17. Return the tube on the magnet for 3 minutes or until the Auto-Mag® D-3 beads are completely cleared from elution buffer.

18. Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at -20°C for long term storage, or for subsequent applications.

Protocol for plant pathogen DNA/plant DNA isolation

The following protocol is for the purification of plant pathogen DNA along with the total plant DNA.

1. Disrupt the plant tissue：

(1) Disrupt the sample mechanically

Plant tissue can be homogenized with a rotor-stator, bead mill, or high-throughput homogenizers. We recommend bead mill or high-throughput homogenizers paired with stainless steel beads. When using beads for homogenization, ensure that the correct tubes are used, which typically have thicker walls and a screw cap with an O-ring.

- Place 30-50mg of fresh plant tissue or 10–20 mg of seed samples into 2.0 ml Homogenizer Bead Beating Tubes in the presence of one or two grinding beads.
- Grind sample using a mechanical grinder following the manufacturer's instructions.
- After sample homogenization, go to step 2

(2) Disrupt the sample manually:

- Place 30-50mg of fresh plant tissue or 10–20 mg of seed samples into a mortar that contains liquid nitrogen,
- Grind the tissue thoroughly using a clean pestle, then allow the liquid nitrogen to evaporate
- Transfer the powder to a Nuclease-free 1.5 ml microcentrifuge tube and go to step 2

2. For lysis pathogen, add 500µl PDL Buffer and 20µl LEM-1, vortex at maximum speed for 20 seconds or pipette mix 20 times.

   Note: If there are clumps of plant matter on the top of the sample tube, please pulse centrifugation to ensure the plant matter is covered by the PDL Buffer. LEM-1 is specially for lysis bacteria/pathogen.

3. Incubate the sample at 37ºC for 10 minutes. Occasionally mix the sample 1-2 times during incubation.

4. Add 50µl of PLE-1 and vortex at maximum speed for 20 seconds. Incubate the sample at 70ºC for 30 minutes. Occasionally mix the sample 2-3 times during incubation.

5. Cool sample at room temperature. Add 200µl of IHR reagent and vortex at maximum speed for 20 seconds. Incubate samples at room temperature for 2 minutes.

   Note: Completely resuspend IHR Reagent by shaking or vortexing the bottle before use. If necessary, use wide-bore tips (or cut the tips to form a wider tip) for the ease of pipetting of the IHR Reagent.

6. Centrifuge the sample tube at ≥ 16,000g at room temperature for 10 minutes to pellet the undigested debris. Collect and transfer ~400 µl of the supernatant to a new microcentrifuge tube.

   Note: Use centrifuge for microcentrifuge tubes. Do not transfer any pellet or other debris.

7. Add 400µl PDB Buffer, 200µl 100% isopropanol and 10µl Auto-Mag® D-3, vortex at maximum speed for 20 seconds or pipette mix 20 times. Incubate the sample tube at room temperature for 8 minutes.

Note: PDB Buffer, isopropanol and Auto-Mag® D-3 can be prepared as a master mix prior to use. Prepare only what is needed and mix completely. Complete resuspension of the Auto-Mag® D-3 beads are crucial for obtaining purity.

8. Place the samples tube on a compatible magnetic separation device for 3 minutes or until Auto-Mag® D-3 beads are completely cleared from the solution. Remove and discard all the liquid. Do not disturb the attracted beads.

9. Remove the sample tube from the magnet. Add 600µl MDW Buffer and resuspend the Auto-Mag® D-3 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times.

   Note: MDW Buffer must be diluted with ethanol prior to use. Complete resuspension of the magnetic beads is critical for obtaining good purity DNA.

10. Place the sample tube on the magnet for 3 minutes or until Auto-Mag® D-3 beads are completely cleared from the solution. Remove and discard all the liquid. Do not disturb the attracted beads.

11. Repeat Steps 9-10 for second MDW wash.

12. Remove the sample tube from the magnet. Add 600µl of freshly prepared 70% ethanol and resuspend the Auto-Mag® D-3 beads by vortex at maximum speed for 20 seconds or pipette mix 20 times.

13. Place the sample tube on the magnet for 5 minutes or until Auto-Mag® D-3 beads are completely cleared from the solution. Remove and discard all the liquid. Do not disturb the attracted beads.

14. Repeat Steps 12-13 for second 70% ethanol wash.

15. Keep the sample tube on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

    Note: *It is critical to completely remove all liquid from each tube.*

16. Add 50~100µl DNA Elution Buffer to the sample and resuspend the Auto-Mag® D-3 beads by vortex for 20 seconds or pipette mix 20 times. Incubate the sample tube at 65°C for 5 minutes.

17. Place the sample tube back on the magnet for 3 minutes or until the Auto-Mag® D-3 beads are completely cleared from elution buffer.

18. Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at -20°C for long term storage, or for subsequent applications.

Protocol and Programmed Procedure for Automation Purification

Please contact technical support via: Phone: 404-290-5063 (in US), Email: support@amdbiotech.com

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via: Phone: 404-290-5063 (in US), Email: support@amdbiotech.com

Symptoms	Possible Causes	Comments
Low DNA Yields	Incomplete disruption of starting material	Make sure to grind the samples completely
	Poor lysis of tissue	Decrease the amount of starting material
	DNA remains bound to Auto-Mag® D-3 beads	Increase elution volume to 100µl and incubate the sample at 65°C for 5 minutes before eluting
	Incomplete resuspension of Auto-Mag® D-3 beads	Increase pipette mixing to resuspend the Auto-Mag® D-3.
	Loss of Auto-Mag® D-3 beads during operation	Avoid disturbing the Auto-Mag® D-3 beads during aspiration of supernatant
	Ethanol is not added into MDW Buffer	Add absolute 100% Ethanol to MDW (see Page 3 for instructions)
Auto-Mag® D-3 beads do not completely clear from solution	Too short of magnetizing time	Increase collection time on the magnet.
Problems in downstream applications	Ethanol carry-over	Dry the Auto-Mag® D-3 completely before elution

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® Plant DNA Plus Kit	D018-00 D018-01 D018-02 D018-Bulk	5 Preps. 96 Preps. 384 Preps. Request
Prefilled Tissue Disruptor Tube (RNase free)	B014-00 B014-01 B014-02 B014-Bulk	5 100 200 Request

AMD Biotech is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call: 404-290-5063

Trademarks

The trademarks mentioned herein are the property of AMD Biotech Inc. or their respective owners.