AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® Plant DNA CTAB Kit

Version 2.1

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

Catalog Number: D022-00, D022-01, D022-02,

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 CTAB Kit uses Auto-Mag® magnetic beads technology and is specially designed for rapid and reliable purification of high-quality genomic DNA from a wide variety of plant samples. Auto-Mag® Plant DNA CTAB Kit efficiently eliminates polysaccharides, phenolic compounds, and enzyme inhibitors with no organic extractions. Purified genomic DNA is suitable for various conventional operations, including enzyme digestion, PCR, Next Generation Sequencing, Southern blot, chip detection, hybridization applications and other experiments. The protocol 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 gDNA from a wide variety of plant samples.
- Highly specific binding of DNA allows easy removal of impurities and inhibitors.
- Eluted DNA is suitable for most downstream applications.
- Automation-friendly

Kit Contents

Product Number	D022-00	D022-01	D022-02
Preparation	5	96	384
Auto-Mag® D-3	0.06 ml	1.1.ml	4.4 ml
CTAB Lysis Buffer	4 ml	60 ml	240 ml
Binding Buffer	3 ml	55 ml	220 ml
MDW Buffer*	5 ml	60 ml	120 ml x2
DNA Elution Buffer	1 ml	20 ml	80 ml
RNase A	0.03 ml	0.55 ml	2.2 ml
* Ethanol must be added prior to use. See Preparation of Reagents			

Storage and Stability

Auto-Mag® Plant DNA CTAB Kit is shipped at ambient temperature. All components are stable for 12 months when stored accordingly. 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. 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. Dilute MDW Buffer with 100% Ethanol as follows and store at room temperature.

Reagents	Kit	100% Ethanol to be Added
MDW Buffer	D022-00	5 ml
	D022-01	60 ml
	D022-02	120 ml per bottle
Components are stable for one year when stored closed at room temperature		

2. Freshly prepared 70% ethanol for DNA wash steps and prepare at least 1.6 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	50-150 µl
Processing format	Auto, Manual
Downstream Application	NGS, PCR, qPCR, real-time RT-PCR, and Sequencing etc.

Auto-Mag® Plant DNA CTAB Method 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 Benchtop vortex mixer
- 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 65°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 65°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 600µl CTAB Lysis Buffer and 5µl RNase A to each sample. Mix by pipetting 20 times or vortex for 20 seconds.

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

3. Incubate the sample at 65ºC for 30 minutes. Occasionally mix the sample 1-2 times during incubation.

4. Centrifuge the sample tube at 16,000g x g for 10 min. Collect and transfer 300µl cleared lysate to a new tube.

   Note: Do not disturb the pellet or transfer any debris as that can reduce the yield.

5. Add 500µl Binding Buffer, 100µl 100% Isopropanol and 10µl Auto-Mag® D-3 to each sample, and pipette mix 20 times or vortex for 20 seconds. Incubate the sample tube at room temperature for 8 minutes.

   Note: Complete resuspension of the Auto-Mag® D-3 beads are crucial to obtain purity. Binding 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.

6. 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.

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

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

8. 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.

9. Repeat Steps 7-8 for second MDW wash.

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

11. 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.

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

13. 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.*

14. Remove the tube off the magnetic separation device, add 50-150µl 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.

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

16. 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 96-well plate

1. Disrupt the plant tissue：

   To prepare samples in 96-well plate format, place samples in a sealed 96-well deep-well plate or capped microtube rack in the presence of one or two grinding beads. Process in the Mixer Mill MM 300 (Retsch), Gino/Grinder® (SpexSamplePrep), or a FastPrep-96™ (MP Biomedicals)

   - Place 30-50mg of fresh plant tissue or 10–20 mg of seed samples in 96 well deep plate.
   - Seal the plate with a mat and process it in a bead disruption mill according to manufacturer's instructions.
   - After sample homogenization, go to step 2.

2. Add 600µl CTAB Lysis Buffer and 5µl RNase A to each sample well. Mix by pipetting 20 times or vortex for 20 seconds.

   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 CTAB Lysis Buffer.

3. Seal the plate and incubate the processing plate at 65ºC for 30 minutes.

4. Centrifuge the plate at 4,000 x g for 10 min. Collect and transfer 300µl cleared lysate to a new 96-well plate.

   Note: Do not disturb the pellet or transfer any debris as it can reduce the yield.

5. Add 500µl Binding Buffer, 100µl 100% Isopropanol and 10µl Auto-Mag® D-3 to each sample, and pipette mix 20 times or vortex for 20 seconds. Incubate the sample tube at room temperature for 8 minutes.

   Note: Complete resuspension of the Auto-Mag® D-3 beads are crucial to obtain purity. Binding 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.

6. Place the samples plate 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.

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

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

8. Place the sample plate 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.

9. Repeat Steps 7-8 for second MDW wash.

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

11. Place the sample plate 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.

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

13. Keep the sample plate 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.*

14. Remove the plate off the magnetic separation device, add 50-150µl 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.

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

16. 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 Automated Isolation Process

If automating these procedures on a liquid handler or a magnetic processor, please contact AMD Biotech for instrument-specific instructions and additional processing procedures.

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	D022-00 D022-01 D022-02 D022-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