Auto-Mag® Plant DNA CTAB Kit

Version 3.0

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

Highlights:

- Efficiently isolates high-quality DNA from a wide variety of plant samples.
- Supports manual, automated, and optional centrifugation-based workflows for flexible and cost-effective sample processing.

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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

- Isolates high-quality gDNA from a wide range of plant samples.
- Highly specific DNA binding enables efficient removal of impurities and inhibitors.
- Eluted DNA is suitable for a wide variety of downstream applications.
- Compatible with both manual and automated processing; cost-effective solution.

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	4 ml	60 ml	240 ml
MDW Buffer*	5 ml	60 ml	120 ml x2
DNA Elution Buffer	1 ml	20 ml	80 ml
RNase A	0.03 ml	0.5 ml	2.0 ml
* Ethanol must be added prior to use. See Preparation of Reagents			

Storage and Stability

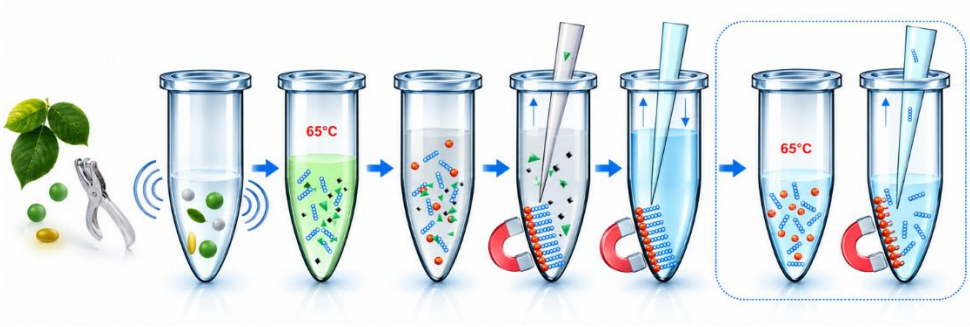
Auto-Mag® Plant DNA CTAB Kit is shipped at an ambitious temperature. All components are stable for 12 months when stored accordingly. Auto-Mag® D-3, and RNase A can be stored at room temperature (15-25°C) for 12 months, to prolong the shelf-life, storage at 2-8°C is recommended. All other components 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 them.

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.

2. Overview of the Workflow for Plant DNA Isolation



1. Disrupt and Lysis plant samples.
2. Add beads to bind nucleic acids.
3. Separate the beads from contaminants
4. Wash magnetic to remove contaminants.
5. Elute DNA from magnetic beads.

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 (AMD: Cat# B092)
- Equipment for disrupting plant tissue (Geno/Grinder, Mixer Mill, or Bead Mill Homogenizers etc.)
- Single-tube format: Nuclease-free 1.5-2.0 ml microcentrifuge tube, and Magnetic Rack Separator for microcentrifuge tube.
- 96-well format: 1.2 ml deep-well plate. Compatible magnetic separation device for 96-well plate
- Sealing film for 96-well plate
- 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.
- 100% Ethanol

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	Follow bottle instruction
	D022-01	60 ml
	D022-02	120 ml per bottle
Components are stable for one year when stored closed at room temperature		

2. 80% Ethanol:

Freshly prepare 80% ethanol using deionized water and 100% ethanol. Mix thoroughly and store at room temperature. Prepare at least 1.6 mL per sample for the final cfDNA wash steps.

3. The Binding Buffer contains a small amount of isopropanol; ensure the cap is tightly closed after each use.

Before Starting

- Please read this protocol in its entirety to become familiar with the procedures
- Prepare reagents according to instructions of Preparation Reagents
- Prepare 80% ethanol for DNA wash steps
- Re-dissolve any precipitates of CTAB Lysis Buffer by warming to 37˚C
- Preset water bath, incubator, or heating blocks to 65°C
- Fully resuspend Auto-Mag® D-3 Bead by vortexing before use.

Protocol for single-tube 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 the 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 of CTAB Lysis Buffer and 5µl of RNase A to each sample. Mix thoroughly by pipetting 20 times or vortex for 20 seconds.

   Note: If clumps of plant material remain on the top of the sample tube, briefly pulse centrifuge to ensure that all plant material is fully submerged in 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 minutes. Carefully transfer 300–400µl of the cleared lysate to a new tube.

   Note: Avoid disturbing the pellet or transferring any debris, as this may reduce DNA yield.

5. Add 600µl of Binding Buffer and 10µl of Auto-Mag® D-3 to each sample. Mix thoroughly by pipetting 20 times, or vortex for 20 seconds.

   Note: Complete resuspension of the Auto-Mag® D-3 beads are crucial to obtain purity.
   The Binding Buffer and Auto-Mag® D-3 may be prepared as a master mix immediately before use. Prepare only the amount needed and mix completely.

6. Incubate the sample at room temperature for 10 minutes.

7. Place the sample tube on a compatible magnetic separation device for 5 minutes, or until the Auto-Mag® D-3 beads are completely cleared from the solution. Remove and discard all liquid carefully without disturbing the beads.

   Note: Some samples may appear dark in color, making it difficult to visually confirm complete magnetic bead separation. Therefore, a 5-minute magnetic separation step is required.

8. Remove the sample tube from the magnet. Add 600µl of MDW Buffer and thoroughly resuspend the beads by pipetting 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 high-purity DNA.

9. Place the tube on the magnet for 3 minutes or until the beads are fully cleared from the solution. Carefully remove and discard the liquid without disturbing the beads.

10. Repeat Steps 8-9 for a second MDW buffer wash.

11. Remove the sample tube from the magnet. Add 600µl of freshly prepared 80% ethanol and thoroughly resuspend the beads by pipetting 20 times or vortex for 20 seconds.

12. Place the tube on the magnet for 3 minutes or until the beads are completely cleared from the solution. Carefully remove and discard the liquid without disturbing the beads.

13. Repeat Steps 11-12 for a second 80% ethanol wash.

14. 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 essential to completely remove all residual liquid from each well.*

15. Remove the tube from the magnet. Add 50–100µl of Elution Buffer to each sample and thoroughly resuspend the Auto-Mag® D-3 beads by pipetting 20 times or vortex for 20 seconds.

16. Incubate the samples at 65°C for 5 minutes.

17. Return the tube on the magnet for 5 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 Store at –20°C for long-term preservation or use immediately for downstream applications.

Protocol for 96-well plate format

1. Disrupt the plant tissue：

   To prepare samples in a 96-well plate format, place the samples in a sealed 96-well deep-well plate or capped microtube rack along with one or two grinding beads. Homogenize the tissue using a Mixer Mill MM 300 (Retsch), Gino/Grinder® (SpexSamplePrep), or FastPrep-96™ (MP Biomedicals) according to the manufacturer's instructions.

   - 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 the sample homogenization, go to step 2.

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

   Note: If clumps of plant material remain on the well walls or surface, briefly pulse centrifuge to ensure that all plant material is fully submerged in the CTAB Lysis Buffer.

3. Seal the plate and incubate the plate at 65ºC for 30 minutes. Occasionally mix the sample 1-2 times during incubation.

4. Centrifuge the plate at 4,000 × g for 10 minutes. Carefully transfer 300–400µl of the cleared lysate to a new 96-well plate.

   Note: Avoid disturbing the pellet or transferring any debris, as this may reduce DNA yield.

5. Add 600µl of Binding Buffer and 10µl of Auto-Mag® D-3 to each sample. Mix thoroughly by pipetting 20 times, or seal the plate and vortex for 20 seconds.

   Note: Complete resuspension of the Auto-Mag® D-3 beads are crucial to obtain purity.
   The Binding Buffer and Auto-Mag® D-3 may be prepared as a master mix immediately before use. Prepare only the amount needed and mix completely.

6. Incubate the sample at room temperature for 10 minutes.

7. Place the sample plate on a compatible magnetic separation device for 5 minutes, or until the Auto-Mag® D-3 beads are completely cleared from the solution. Remove and discard all liquid carefully without disturbing the beads.

   Note: Some samples may appear dark in color, making it difficult to visually confirm complete magnetic bead separation. Therefore, a 5-minute magnetic separation step is required.

8. Remove the sample plate from the magnet. Add 600µl of MDW Buffer and thoroughly resuspend the beads by pipetting 20 times or seal the plate and 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 high-purity DNA.

9. Place the plate on the magnet for 3 minutes or until the beads are fully cleared from the solution. Carefully remove and discard the liquid without disturbing the beads.

10. Repeat Steps 8-9 for a second MDW buffer wash.

11. Remove the sample plate from the magnet. Add 600µl of freshly prepared 80% ethanol and thoroughly resuspend the beads by pipetting 20 times or seal the plate and vortex for 20 seconds.

12. Place the plate on the magnet for 3 minutes or until the beads are completely cleared from the solution. Carefully remove and discard the liquid without disturbing the beads.

13. Repeat Steps 11-12 for a second 80% ethanol wash.

14. 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 essential to completely remove all residual liquid from each well.*

15. Remove the plate from the magnet. Add 50–150µl of Elution Buffer to each well and thoroughly resuspend the Auto-Mag® D-3 beads by pipetting 20 times or seal the plate and vortex for 20 seconds.

16. Incubate the samples at 65°C for 5 minutes.

17. Return the plate on the magnet for 5 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 Store at –20°C for long-term preservation or use immediately for downstream applications.

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 CTAB Kit	D022-00 D022-01 D022-02 D022-Bulk	5 Preps. 96 Preps. 384 Preps. Request
Auto-Mag® Prefilled Disruptor Tube, 2.0 mL capacity (4.5 mm Steel Beads)	B092-50 B092-Bulk	50 Request

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