Axygen® AxyPrep™ Mag Plant gDNA Kit

Protocol

A Corning Brand

Introduction

The AxyPrep Mag Plant Genomic DNA (gDNA) kit is a magnetic bead-based purification system that allows rapid and reliable isolation of high quality gDNA from a wide variety of plant samples. Up to 96 samples (50 mg fresh tissue per sample, or 15 mg of dried tissue per sample) can be processed in less than one hour. This kit efficiently eliminates polysaccharides, phenolic compound and enzyme inhibitors with no organic extractions. The kit is adaptable on major liquid handling workstations for high throughput processing.

This method will produce gDNA suitable for PCR and qPCR. Because of the high throughput processing method, high molecular weight gDNA may be sheared during lysis and may not be suitable for certain hybridization-based assays or Southern blotting.

Process Overview

1. Plant samples and lysis buffer are combined and processed in a homogenizer/bead based milling equipment.
2. Supernatant is transferred to a new processing plate where **MAG-A1 Particles** are added to bind to the DNA.
3. Following a few wash steps, DNA is eluted from the **MAG-A1 Particles** for downstream application.

Materials Supplied in the Kit

- **MAG-A1 Particles:** Magnetic beads. Store at 2 to 8°C upon arrival, for up to 12 months. DO NOT FREEZE.
- **PCL Buffer:** Lysis buffer. Store at room temperature for up to 12 months.
- **PDB Buffer:** Binding buffer. Store at room temperature for up to 12 months.
- **HSW Buffer:** Wash buffer. Store at room temperature for up to 12 months.
- **MB Elution Buffer:** Elution buffer. Store at room temperature for up to 12 months.

All components are stable for a period of at least 12 months from the date of manufacture when stored accordingly. Avoid exposure to direct sunlight or temperature extremes.

Caution

All components are stable for a period of at least 12 months from the date of manufacture when stored accordingly. Avoid exposure to direct sunlight or extreme temperatures. When working with buffers, always wear suitable protective equipment such as safety glasses, laboratory coat and gloves. Be careful to avoid contact with skin and eyes. In the case of such contact, wash immediately with water. If necessary, seek medical assistance. For more information, please consult the safety datasheet (SDS).

Hardware and Consumables

Cat. No.	MAG-PLANT-gDNA-10	MAG-PLANT-gDNA-S	MAG-PLANT-gDNA-M
Kit Size	10 preps	96 preps	384 preps
MAG-A1 Particles	250 µL	2.2 mL	10 mL
PCL Buffer	8 mL	60 mL	250 mL
PDB Buffer	7 mL	60 mL	250 mL
HSW Buffer	4 mL	44 mL	100 mL
MB Elution Buffer	1.5 mL	15 mL	60 mL
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Materials to Be Supplied by the User

- Equipment for disrupting plant tissues (bead based milling with beads)
- Benchtop microcentrifuge capable of 12,000 xg, such as Corning® LSE™ High Speed Microcentrifuge (Corning Cat. No. 6765-HS) with a rotor adaptor for 96 well deep well plate
- Magnetic separation device for 96 well plate (Axygen Cat. No. IMAG-96P)
- 96 well deep well plates compatible with the magnetic bead separation device
- Sealing mat for 96 well deep plates
- Incubator capable of 65°C
- Reagent reservoirs
- 70% ethanol
- 100% ethanol
- Isopropanol (100%)
- RNase A (10 mg/mL)

Preparation Before Experiment

1. Add the volume of ethanol specified below to HSW Buffer and mix well. Use 100% ethanol. Indicate on the bottle that this has been completed.

Catalog No.	Component	Add 100% Ethanol	Storage
MAG-PLANT-gDNA-10	HSW Buffer	5 mL	15 to 25°C
MAG-PLANT-gDNA-S	HSW Buffer	56 mL	15 to 25°C
MAG-PLANT-gDNA-M	HSW Buffer	224 mL	15 to 25°C

2. Pre-warming MB Elution Buffer to 55°C may improve elution efficiency.

3. Set incubator to 65°C.

Protocol for AxyPrep™ Mag Plant Genomic DNA Kit

1. Binding Step
   a. Place up to 50 mg fresh leaf disks or up to 15 mg dried tissue in a 96 well deep well plate in the presence of one or two grinding beads.
   b. Seal the plate with a mat and process in a bead disruption mill. Follow the manufacturer's instructions.
   c. Add 600 µL **PCL Buffer** and 2 µL RNase A to the sample.

      Optional: PCL Buffer and RNase A can be prepared as master mix.
   d. Seal the plate and incubate the processing plate at 65°C for 15 minutes. Spin in a centrifuge at 4,000 xg for 10 minutes.
   e. Tap the plate and remove the seal from the plate. Carefully aspirate 300 µL cleared lysate to a new 96 well plate.

      Note: Do not distrub the pellet or transfer any debris as it may reduce the yield and purity.
   f. Add 600 µL **PDB Buffer** and 20 µL **MAG-A1 Particles.** Pipet mix 10 times.

      Note: Shake **MAG-A1 Particles** thoroughly to fully resuspend before use.
   g. Incubate the sample plate at room tempertaure for 5 minutes. Mix once by pipet mixing.
   h. Place the sample processing plate on the magnetic separation device to separate the **MAG-A1 Particles** for 5 minutes or until the beads clear from the solution.
   i. With the plate on the magnetic separation device, carefully pipet supernatant and discard.

      Note: Do not disturb the attracted beads while aspirating the supernatant.
2. Washing Step
   a. Remove the plate from the magnetic separation device, add 400 µL **HSW Buffer** to each sample and pipet mix 10 times to resuspend the **MAG-A1 Particles**.
   b. Place the sample processing plate on the magnetic separation device to separate the **MAG-A1 Particles** for 5 minutes or until the beads clear from the solution.
   c. With the plate on the magnetic separation device, carefully pipet supernatant and discard.

      Note: Do not disturb the attracted beads while aspirating the supernatant.
   d. Repeat steps 2a to 2c for a second wash.
   e. Remove the plate off the magnetic separation device, add 400 µL 70% ethanol to each sample and pipet mix 10 times to resuspend the **MAG-A1 Particles**.
   f. Place the sample processing plate on the magnetic separation device to separate the **MAG-A1 Particles** for 5 minutes or until the beads clear from the solution.
   g. With the plate on the magnetic separation device, remove and carefully pipet supernatant and discard.

      Note: Do not disturb the attracted beads while aspirating the supernatant.
   h. Remove the plate off the magnetic separation device, add 400 µL 100% ethanol to each sample and pipet mix 10 times to resuspend the **MAG-A1 Particles**.
   i. Place the sample processing plate on the magnetic separation device to separate the **MAG-A1 Particles** for 5 minutes or until the beads clear from the solution.

      Note: Do not disturb the attracted beads while aspirating the supernatant.
   j. With the plate on the magnetic separation device, carefully pipet supernatant and discard.
   k. Incubate the beads for 10 minutes at room temperature with the plate still attached to the magnetic separation device.

3. Elution Buffer

   a. Remove the plate off the magnetic separation device, add 50 to 100 µL **MB Elution Buffer** to the sample and pipet mix 15 times to resuspend the **MAG-A1 Particles**.

      Note: Pre-warming the **MB Elution Buffer** to 55°C will often improve elution efficiency.

   b. Incubate the sample plate at room temperature for 5 minutes.

   c. Place the sample processing plate on the magnetic separation device to magnetize the **MAG-A1 Particles** for 5 minutes or until the beads clear from the solution

   d. Transfer the eluate (cleared supernatant containing the DNA) to a new plate. Store DNA at -20°C.

Warranty/Disclaimer

AxyPrep™ kits are designed for research use only. Corning Life Sciences makes no claims regarding the performance of these kits for clinical or diagnostic applications. Corning warrants that this kit will conform to Corning's specifications for this kit for a period of up to 12 months from the date of manufacture when stored in the specified manner and used according to the instructions provided. In using this product, the customer agrees that Corning shall not be liable to the customer or any other party for any direct or indirect damages, including, but not limited to personal injury, property damage, special, consequential, indirect or punitive damages or lost profits (or other economic loss) resulting from the use or inability to use this product, even if such losses or damages are reasonably foreseeable.

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