MAGBIO

Liquid Biopsy Research - “We are where you start”

www.magbiogenomics.com

MAGBIO

Liquid Biopsy Research - “We are where you start”

HighPrep™ Plant DNA Plus Kit

Catalog Nos. HPPP-D5, HPPP-D50, HPPP-D100, HPPP-D100X4

Manual Revision

v1.0

- Genomic DNA Isolation from a variety of plant species
- Magnetic-beads based chemistry

PROTOCOL

Contents

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

Product Description

The HighPrep™ Plant DNA Plus Kit is specially designed for purifying DNA from a wide range of plant and fungi species. The kit uses a special lysis condition with HighPrep™ magnetic particles technology to isolate high-quality genomic DNA. Under DNA binding conditions, only genomic DNA binds to the magnetic particles while most of the contaminating cellular proteinaceous components are removed. The purified DNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, Southern blotting, SNP analysis, NGS, etc. The HighPrep™ Plant DNA Plus Kit can be adapted to high-throughput liquid handling work stations.

Advantages

• Fast and easy processing using a magnetic bead system.
• Robust lysis system (chemical lysis combined with a mechanical homogenization).
• High yield - consistent, high yield of inhibitor-free DNA up to 50 kb plus.
• Isolate high-quality total DNA from a variety of plant species, including any pathogen DNA present.

Process

Plant samples are disrupted in a homogenizer/bead-based milling equipment. LB Buffer is added to lyse the samples. The supernatant is then transferred to a new processing plate where MAG-G Particles are added to bind to the DNA. Following a few wash steps, DNA is eluted from MAG-G Particles for downstream application.

Kit Contents and Storage

HighPrep™ Plant DNA Plus Kit Catalog No.	HPPP-D5	HPPP-D50	HPPP-D100	HPPP-D100X4	STORAGE
Number of Preps	5	50	100	400	
MAG-G Particles	0.06 mL	.55 mL	1.1 mL	4.4 mL	2-8°C
LB Buffer	2.5 mL	22 mL	42 mL	170 mL	15-25°C
DB Buffer	2.5 mL	22 mL	42 mL	170 mL	15-25°C
DW Buffer[1]	1.6 mL	16 mL	32 mL	128 mL	15-25°C
DWB Buffer[1]	2 mL	18 mL	36 mL	144 mL (72mL x	15-25°C
Elution Buffer	1 mL	8 mL	15 mL	60 mL	15-25°C
Pro K Solution	0.11 mL	1.1 mL	2.1 mL	8.5 mL	2-8°C
RNase A	0.026 mL	.26 mL	0.52 mL	2.1 mL	2-8°C

[1] Ethanol must be added prior to use. See Preparation of Reagents

HighPrep™ Plant DNA

MagBio Genomics, Inc.

Stability

All components are stable for 14 months when stored accordingly.

Safety Information

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 Resource” tab when viewing the product kit.

HighPrep™ Plant DNA

MagBio Genomics, Inc.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add 100 % Ethanol	Storage
HPPP-D5	DW Buffer	2 mL	Room Temp 15-25°C
	DWB Buffer	5 mL	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Catalog No.	Component	Add 100 % Ethanol	Storage
HPPP-D50	DW Buffer	20 mL	Room Temp 15-25°C
	DWB Buffer	45 mL	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Catalog No.	Component	Add 100 % Ethanol	Storage
HPPP-D100	DW Buffer	40 mL	Room Temp 15-25°C
	DWB Buffer	90 mL	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Catalog No.	Component	Add 100 % Ethanol	Storage
HPPP-D100X4	DW Buffer	160 mL	Room Temp 15-25°C
	DWB Buffer	180 mL per bottle	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Amounts of starting material

Use the amounts of starting material indicated in Table 1.

Table 1. Amounts of starting material for HighPrep™ Plant DNA Plus kit

Sample	Amount
Plant Tissue	up to 50 mg

HighPrep™ Plant DNA Plus Kit - Fresh/Frozen Samples

Equipment and Reagents to Be Supplied by User

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) from each product supplier.

☐ Nuclease-free ~2 ml microcentrifuge
☐ tubes. Magnetic separation device.
☐ Water bath, incubator, or heating block capable of 65°C.
☐ Equipment for disrupting plant tissue (Geno/Grinder 2010 or MM300 Mixer Mill etc).
☐ 100%
☐ Ethanol.
Vortex.

Things to do before starting

☐ Prepare DW and DWB according to the instructions in the Preparation of Reagents
☐ Section. Preset water bath, incubator, or heating block to 65°C.
☐ Preheat Elution Buffer to 65°C.
☐ Suspend MAG-G Particles by vortexing.

Protocol

1. **Grinding plant sample:**

   1) Homogenization method: Place ≤50 mg of plant tissue into a capped microtube in the presence of one or two grinding beads and add 400 µl of LB Buffer. Process in the MM300
   Mixture Mill or Geno/Grinder Mixture Mill following the manufacturer's instructions to grind sample (example: 4000rpm, 90 seconds X 2).

   2) Place ≤50 mg of plant tissue into a mortar that contains liquid nitrogen then grind into a powder. Transfer the plant or fungi powder to a DNase-free 1.7 mL microcentrifuge tube and add 400 µl LB Buffer.

2. **Add 20 ul of Proteinase K (20mg/ml) and vortex to mix thoroughly. Incubate at 65°C for 20 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.**

3. **Add 400 µl of DB Buffer, mix by vortexing the tube, and incubate for 5 minutes at room temperature.**

4. **Centrifuge the tube for 10 minutes at ~20,000 × g (~14,000 RPM).**

MagBio Genomics,
Inc.

5. **Using a pipette, transfer up to 400 µl of supernatant (avoid contacting the pellet with the pipette tip) into a 2 ml DNase-free microcentrifuge tube (not provided).**

   ⚠ Optional: If RNA-free gDNA is required, add 5 µl of RNase A and incubate for 5 minutes at room temperature.

6. **Add 400 µl of 100% Ethanol (provided by the user) and 10 µl of MAG-G Particles (vortex prior to use) to the lysate collected above.**

7. **Mix the samples by pipetting 20 times or vortexing for 20 seconds. Incubate at room temperature for 10 minutes and briefly mix the samples several times during incubation.**

8. **Place the tubes back on the magnetic separation device and wait 2-5 minutes or until the magnetic particles are completely cleared from the solution. Aspirate and discard the cleared supernatant. Do not disturb the MAG-G Particles.**

9. **Add 600µl of DW buffer to each sample. Re-suspend the MAG-G Particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times.**

   ⚠ Note: DW buffer must be diluted with Ethanol before use.

10. **Place the tubes back on the magnetic separation device and wait 2-5 minutes or until the magnetic particles are completely cleared from the solution. Aspirate and discard the cleared supernatant. Do not disturb the MAG-G Particles.**

11. **Add 600µl of DWB buffer to each sample. Re-suspend the MAG-G Particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times.**

    ⚠ Note: DWB buffer must be diluted with Ethanol before use.

12. **Place the tubes back on the magnetic separation device and wait 2-5 minutes or until the magnetic particles are completely cleared from the solution. Aspirate and discard the cleared supernatant. Do not disturb the MAG-G Particles.**

13. **Repeat Steps 11-12 for a second DNA wash step.**

14. **Leave the tubes on the magnetic separation device for 5-10 minutes to air dry the MAG-G Particles. Remove any residual liquid with a pipette.**

    ⚠ Note: it is critical to completely remove all liquid from each tube.

15. **Add 50-100 µl of Elution Buffer and completely resuspend the MAG-G Particles by vortexing at maximum speed. Incubate at 65°C for 5 minutes.**

16. **Place the tubes on a magnetic separation device to magnetize the magnetic particles for 5 minutes or until the MAG-G Particles are completely cleared from the elution buffer.**

17. **Transfer the cleared supernatant containing DNA to a new 1.5 ml tube. Store the DNA at -20°C.**

Troubleshooting guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: 1-855-262-4246 (in US), outside US, 1-919-719-0665
Email: support@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low DNA yield	Incomplete disruption of starting material	Make sure to grind samples completely.
	Poor lysis of tissue	Decrease amount of starting material.
	DNA remains bound to MAG-G Particles	Increase elution volume to 100 µl and incubate at 65°C for 5 min before
	Incomplete resuspension of MAG-G Particles	Increase pipette mixing to resuspend the MAG-G Particles.
	Loss of MAG-G Particles during	Avoid disturbing the MAG-G Particles during aspiration of
	Ethanol is not added into DW and DWB Buffer	Add absolute 100% ethanol to DW and DWB Buffer (see page 2 for instructions).
MAG-G Particles 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 MAG-G Particles completely before elution.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves, and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs).