

## MAGneat Plant gDNA Extraction Kit

MN-PLT-96

96 Extractions

Manual Date: December 2025

Version Number: 3.0

**Location:** Microzone,  
Suite 3, Faraday House,  
King William Street,  
Stourbridge,  
DY8 4HD

**T:** +44(0)1384 444585

**E:** [info@microzone.co.uk](mailto:info@microzone.co.uk)

**W:** [microzone.co.uk](http://microzone.co.uk)

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## Introduction

The **MAGneat Plant gDNA Extraction Kit** is designed for rapid and reliable purification of high-quality genomic DNA from a wide range of plant species. Utilising advanced magnetic bead technology, this kit delivers robust yields of high-molecular-weight DNA suitable for a variety of downstream applications, including PCR, long-read sequencing, restriction enzyme digestion, and other molecular biology workflows.

Plant tissues are known for their challenging composition: rich in polysaccharides, polyphenols, and other secondary metabolites that can interfere with DNA isolation. The MAGneat Kit provides a consistent and efficient solution, minimising such contaminants to produce highly pure DNA. With its high-binding capacity and rapid magnetic response, the system ensures reproducible results and can be easily adapted for automated workflows.

**Important:** If automating this procedure, please contact **Microzone** for guidance and technical support with your specific application.

## Kit Contents

Product	Product Code	MN-PLT-96
Plant Lysis Buffer	MN-PLB-96	71 mL
Proteinase K	MN-PPK-96	1 vial
Plant Precipitation Solution	MN-PPS-96	36 mL
RNase A	MN-PRN-96	1 vial
Plant Binding Buffer	MN-PBB-96	14 mL
MAGneat Magnetic Beads	MN-PMB-96	3 mL
Plant Wash Buffer 1	MN-PWBO-96	28 mL
Plant Elution Buffer	MN-PEB-96	21 mL

## Storage and Stability

Store the MAGneat Plant gDNA Extraction Kit at 4°C until the expiry date displayed on the label. After reconstitution store Proteinase K & RNase A at -20°C.

## Magnetic Separation Devices and Plasticware

Although many brands of magnetic separation devices are compatible with the MAGneat Plant gDNA Extraction Kit, we recommend the MAGneat magnetic separation range from Microzone. These products utilise neodymium magnets to ensure fast separation and defined pellets. Regardless of the magnetic separation device utilised please ensure the device is compatible with the plasticware chosen for use.

## Reagent Preparation

### Solvent Addition

1. Dilute the Plant Binding Buffer with 100% Isopropanol (IPA) as described below and store at 2-8°C.

Product Code	100% IPA to be Added
MN-PBB-96	56 mL

2. Dilute the Plant Wash Buffer 1 with 100% ethanol as described below and store at 2-8°C.

Product Code	100% Ethanol to be Added
MN-PWBO-96	42 mL

3. Make an 80% ethanol solution with molecular grade water, 1.4 mL will be required for each extraction. This will be referred to as **Wash Buffer 2** throughout this manual. Make an excess of 10% to ensure there is sufficient to complete extractions. The table below provides dilution assistance, 10% excess is included in calculations.

Extractions	Molecular Grade Water	100% Ethanol to be Added
5	1.6 mL	6.4 mL
10	3.2 mL	12.8 mL
25	8 mL	32 mL
50	16 mL	64 mL
96	30 mL	120 mL

### Proteinase K Reconstitution

1. Bring proteinase K vial to room temperature.
2. Remove bung and add 2.1 mL of molecular grade water to the proteinase K vial.
3. Replace bung and incubate at room temperature for 30 minutes.
4. Mix well via inversion.
5. Aliquot into required volumes and store at -20°C, avoid multiple free thaw cycles.

**Note:** Ensure that all solutions are at room temperature prior to use.

## RNase A Reconstitution

1. Bring RNase A vial to room temperature.
2. Remove bung and add 2.1 mL of molecular grade water to the RNase A vial.
3. Replace bung and incubate at room temperature for 30 minutes.
4. Mix well via inversion.
5. Aliquot into required volumes and store at  $-20^{\circ}\text{C}$ , avoid multiple free thaw cycles.

**Note:** Ensure that all solutions are at room temperature prior to use.

# Plant gDNA Extraction Protocol – Manually (dry-based homogenisation)

This protocol is suitable for the gDNA extraction of fresh, dried or frozen plant samples using a dry-based homogenisation method to process samples.

## Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- $\beta$ -mercaptoethanol
- Equipment for disrupting plant tissues
- Magnetic separation device
- Vortex
- Heat block or a shaking thermomixer capable of 65°C
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water
- Ice
- Benchtop microcentrifuge

## Preparation

- Prepare the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 as described in the “Solvent Addition” section of page 4.
- Reconstitute the Proteinase K as described in the “Proteinase K reconstitution” section on page 4.
- Reconstitute the RNase A as described in the “RNase A reconstitution” section on page 5.
- Set the heat block to 65°C.
- Check all the solutions in the kit for salt precipitation, especially the Plant Lysis Buffer. If needed, warm solution at 37°C to dissolve any precipitates, then bring to room temperature.

## Procedure

1. Prior to use for sample preparation, prepare a fresh mastermix of Plant Lysis Buffer, Proteinase K and  $\beta$ -mercaptoethanol following the instructions in the below table.

Component	Amount per Prep	Total Amount per 96-well Plate
Plant Lysis Buffer	500 $\mu$ L	50.4 mL*
Proteinase K Solution	20 $\mu$ L	2.02 mL*
$\beta$ -mercaptoethanol	10 $\mu$ L	1.01 mL*

\*5% added for 96-well plate calculations.

2. Weigh up to 50 mg of fresh plant sample or up to 10 mg of dried plant sample and disrupt material with the chosen method: bead mill, mortar and pestle in combination with liquid nitrogen, high-throughput homogeniser, etc.
3. Transfer the homogenised sample to a sterile 2 mL centrifuge tube, add 530  $\mu$ L of mastermix prepared in step 1 and vortex to mix.
4. Incubate samples at 65°C for 30 minutes, vortex vigorously every 10 minutes. Depending on plant species, a longer incubation might be needed.
5. Cool samples down to room temperature and add 350  $\mu$ L of Plant Precipitation Solution.
6. Incubate on ice for 5 minutes.
7. Centrifuge at maximum speed for 5-10 min at room temperature to clear the plant lysate.
8. Transfer 500  $\mu$ L of the supernatant to a clean 2 mL microcentrifuge tube. Do NOT disturb pellet containing cell debris and precipitates.
9. Add 20  $\mu$ L of RNase A (10 mg/mL), mix well by pipetting up/down, and incubate at 37°C for 5-10 minutes.
10. Add 700  $\mu$ L of Plant Binding Buffer and mix well by vortexing until sample is homogenous.

**Note:** Sample may solidify if temperature drops. Warm sample at 37°C to bring it into solution before proceeding to step 11.

11. Add 30 µL of MAGneat magnetic beads to the sample, mix well by vortexing and allow to stand at room temperature for 5 minutes. Mix after 2 minutes 30 seconds by vortexing until the sample is homogenous.

**Note:** Vortex the MAGneat magnetic beads thoroughly ensuring they are in solution prior to use.

12. Transfer the tube to a magnetic separation device for at least 2 minutes or until sample is clear of beads. Then carefully remove and discard the supernatant.

13. Remove the centrifuge tube from the magnetic separation device and add 700 µL of Wash Buffer 1. Mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

14. Transfer the centrifuge tube containing the homogenous sample to the magnetic separation device and magnetise for 1 minute.

15. After 1 minute, carefully remove and discard the clear supernatant.

16. Remove the centrifuge tube from the magnetic separation device and add 700 µL of Wash Buffer 2. Mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

17. Transfer the tube containing the homogenous sample to a magnetic separation device and magnetise for 1 minute.

18. After 1 minute, carefully remove and discard the clear supernatant.

19. Repeat steps 16, 17 and 18 once for a total of 2 washes.

20. Remove the centrifuge tube from the magnetic separation device and let stand at room temperature for 5 to 10 minutes with the cap open to air dry the pellet.

- 21.** During the air-drying step, fluid may accumulate at the bottom of the tube. Use a 20  $\mu$ L pipette to remove any remaining supernatant if required, being careful not to disturb the pellet.
  
- 22.** Add 100 - 200  $\mu$ L of Plant Elution Buffer and vortex until the sample is homogenous, ensuring all beads have been detached from the tube wall.
  
- 23.** Close the cap and incubate at 60°C for 5 minutes.
  
- 24.** Place the tube on a magnetic separation device and magnetise for 2 minutes or until sample is completely clear of beads.
  
- 25.** Carefully transfer the clear supernatant into a new centrifuge tube for downstream processing. Be careful to not disturb the beads.
  
- 26.** If not using immediately, store samples at -20°C for long-term storage or at 2–8°C for up to 24 hours.

# Plant gDNA Extraction Protocol – Manually (wet-based homogenisation)

This protocol is suitable for the gDNA extraction of fresh, dried or frozen plant, using a wet-based homogenisation method to process samples.

## Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- $\beta$ -mercaptoethanol
- Homogeniser Instrument
- Prefilled Homogenisation Tubes
- Magnetic separation device
- Vortex
- Heat block or a shaking thermomixer capable of 65°C
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water
- Ice
- Benchtop microcentrifuge

## Preparation

- Prepare the Plant Binding Buffer, Plant Wash Buffer 1 and Wash Buffer 2 as described in the “Solvent Addition” section of page 4.
- Reconstitute the Proteinase K as described in the “Proteinase K reconstitution” section on page 4.
- Reconstitute the RNase A as described in the “RNase A reconstitution” section on page 5.
- Set the heat block to 65°C.
- Check all the solutions in the kit for salt precipitation, especially the Plant Lysis Buffer. If needed, warm solution at 37°C to dissolve any precipitates, then bring to room temperature.
- Choose an appropriate method to prepare plant samples as extraction is most effective with well homogenised material.
- Yields of DNA may vary depending on sample type, age, and storage conditions.

## Procedure

1. Prior to use for sample preparation, prepare a fresh mastermix of Plant Lysis Buffer, Proteinase K and  $\beta$ -mercaptoethanol following the instructions in the below table.

Component	Amount per Prep	Total Amount per 96-well Plate
Plant Lysis Buffer	700 $\mu$ L	70.56 mL*
Proteinase K Solution	20 $\mu$ L	2.02 mL*
$\beta$ -mercaptoethanol	10 $\mu$ L	1.01 mL*

\*5% added for 96-well plate calculations.

2. Weigh up to 50 mg of fresh plant sample or up to 10 mg of dried plant sample and transfer to homogenisation tubes.
3. Add 730  $\mu$ L of Plant Lysis Buffer, Proteinase K and  $\beta$ -mercaptoethanol mastermix prepared in step 1.
4. Use homogeniser instrument to disrupt the plant material.
5. Centrifuge the homogenate at 2000  $\times$ g until all the foam generated in the homogenisation process is removed and the sample is clear.
6. Transfer 530  $\mu$ L of the homogenate to a 2 mL centrifuge tube.
7. Incubate samples at 65°C for 30 minutes, vortex vigorously every 10 minutes. Depending on plant species, a longer incubation might be needed.
8. Cool samples down to room temperature and add 350  $\mu$ L of Plant Precipitation Solution.
9. Incubate on ice for 5 minutes.
10. Centrifuge at maximum speed for 5 - 10 min at room temperature to clear the plant lysate.

11. Transfer 500  $\mu\text{L}$  of the supernatant to a clean 2 mL microcentrifuge tube. Do NOT disturb pellet containing cell debris and precipitates.
12. Add 20  $\mu\text{L}$  of RNase A (10 mg/mL), mix well by pipetting up/down, and incubate at 37°C for 5-10 minutes.
13. Add 700  $\mu\text{L}$  of Plant Binding Buffer and mix well by vortexing until sample is homogenous.

**Note:** Sample may solidify if temperature drops. Warm sample at 37°C to bring it into solution before proceeding to step 14.

14. Add 30  $\mu\text{L}$  of Plant MAGneat beads to the sample, mix well by vortexing and allow to stand at room temperature for 5 minutes. Mix after 2 minutes 30 seconds by vortexing until the sample is homogenous.

**Note:** Vortex the Plant MAGneat beads thoroughly ensuring they are in solution prior to use.

15. Transfer the tube to a magnetic separation device for at least 2 minutes or until sample is clear of beads. Then carefully remove and discard the supernatant.
16. Remove the centrifuge tube from the magnetic separation device and add 700  $\mu\text{L}$  of Plant Wash Buffer 1. Mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.
17. Transfer the centrifuge tube containing the homogenous sample to the magnetic separation device and magnetise for 1 minute.
18. After 1 minute, carefully remove and discard the clear supernatant.

- 19.** Remove the centrifuge tube from the magnetic separation device and add 700  $\mu\text{L}$  of Wash Buffer 2. Mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.
- 20.** Transfer the tube containing the homogenous sample to a magnetic separation device and magnetise for 1 minute.
- 21.** After 1 minute, carefully remove and discard the clear supernatant.
- 22.** Repeat steps 19, 20 and 21 once for a total of 2 washes.
- 23.** Remove the centrifuge tube from the magnetic separation device and let stand at room temperature for 5 to 10 minutes with the cap open to air dry the pellet.
- 24.** During the air-drying step, fluid may accumulate at the bottom of the tube. Use a 20  $\mu\text{L}$  pipette to remove any remaining supernatant if required, being careful not to disturb the pellet.
- 25.** Add 50 - 150  $\mu\text{L}$  of Plant Elution Buffer and vortex until the sample is homogenous, ensuring all beads have been detached from the tube wall.
- 26.** Close the cap and incubate at 60°C for 5 minutes.
- 27.** Place the tube on a magnetic separation device and magnetise for 2 minutes or until sample is completely clear of beads.
- 28.** Carefully transfer the clear supernatant into a new centrifuge tube for downstream processing. Be careful to not disturb the beads.
- 29.** If not using immediately, store samples at -20°C for long-term storage or at 2–8°C for up to 24 hours.

# Plant gDNA Extraction Protocol – Automated with HeiDi-NA

This protocol is suitable for the gDNA extraction of fresh, dried or frozen plant samples, using HeiDi-NA MAGneat Nucleic Acid Extraction/ Purification System

## Materials and Reagents to be Supplied by User:

- HeiDi-NA MAGneat Nucleic Acid Extraction/ Purification System
- HeiDi-NA 6 Well Sample Strips or HeiDi-NA 96 Deep Well Plates
- HeiDi-NA Magnetic Tip Combs
- 100% ethanol
- 100% isopropanol
- $\beta$ -mercaptoethanol
- Equipment for disrupting plant tissues
- Vortex
- Heat block or a shaking thermomixer capable of 65°C
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water
- Ice
- Benchtop microcentrifuge

## Preparation

- Prepare the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 as described in the “Solvent Addition” section of page 4.
- Reconstitute the Proteinase K as described in the “Proteinase K reconstitution” section on page 4.
- Reconstitute the RNase A as described in the “RNase A reconstitution” section on page 5.
- Set the heat block to 65°C.
- Check all the solutions in the kit for salt precipitation, especially the Plant Lysis Buffer. If needed, warm solution at 37°C to dissolve any precipitates, then bring to room temperature.

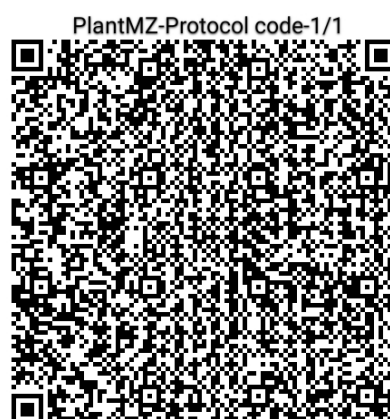
## Procedure

### HeiDi-NA Preparation

1. Pre-dispense the kit's reagents into a HeiDi-NA 6 Well Sample Strips or HeiDi-NA 96 Deep Well Plates, following the table below:

Well Number						
	1	2	3	4	5	6
Component	xx	MAGneat Beads	Wash Buffer 1	Wash Buffer 2	Wash Buffer 2	Elution Buffer
Volume (μL)	xx	30	700	700	700	100 - 200

2. Insert the HeiDi-NA Magnetic Tip Combs into the fixed groove of the magnetic tip comb holder in HeiDi-NA.
3. Using the QR scanner from HeiDi-NA, scan and identify the QR code for the PlantMZ extraction protocol:



### Sample Preparation

1. Prior to use for sample preparation, prepare a fresh mastermix of Lysis Buffer, Proteinase K and β-mercaptoethanol following the instructions in the below table.

Component	Amount per Prep	Total Amount per 96-well Plate
Lysis Buffer	500 μL	50.4 mL*
Proteinase K Solution	20 μL	2.02 mL*
β-mercaptoethanol	10 μL	1.01 mL*

\*5% added for 96-well plate calculations.

2. Weigh up to 50 mg of fresh plant sample or up to 10 mg of dried and disrupt material with the chosen method: bead mill, mortar and pestle in combination with liquid nitrogen, high-throughput homogeniser, etc.
3. Transfer the homogenised sample to a sterile 2 mL centrifuge tube, add 530  $\mu$ L of mastermix prepared in step 1 and vortex to mix.
4. Incubate samples at 65°C for 30 minutes, vortex vigorously every 10 minutes. Depending on plant species, a longer incubation might be needed.
5. Cool samples down to room temperature and add 350  $\mu$ L of Plant Precipitation Solution.
6. Incubate on ice for 5 minutes.
7. Centrifuge at maximum speed for 5-10 min at room temperature to clear the plant lysate.
8. Transfer 500  $\mu$ L of the supernatant to the well number 1 of the prefilled deep 96-well plate/6 well sample strip. Do NOT disturb pellet containing cell debris and precipitates.
9. Add 20  $\mu$ L of RNase A (10 mg/mL) to the sample in well number 1.
10. Place the deep 96-well plate/6 well sample strip into the HeiDi-NA system and close the door.
11. Start the run.
12. At the 'Add Reagent' pause (approximately 5 minutes after initial start), remove plate/strip from the instrument.
13. Add 700  $\mu$ L of Plant Binding Buffer to the sample in well number 1.
14. Place the deep 96-well plate/6 well sample strip back into the HeiDi-NA system, close the door and press 'Confirm' in HeiDi's screen to continue the run.
15. At the end of the run (approximately 30 minutes after initial start), remove the deep 96-well plate/6 well sample strip immediately from the instrument and transfer the eluate (well 6) to a new tube or plate.
16. If not using immediately, store samples at -20°C for long-term storage or at 2–8°C for up to 24 hours.