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MAG PLANT PRO-M GENOMIC DNA EXTRACTION KIT USER GUIDE

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CONTENT

1. DESCRIPTION

The Mag Plant Pro-M Genomic DNA Extraction Kit has been developed to extract genomic DNA from a wide variety of fresh or frozen plant tissues, delivering high yields and purity. The method is based on the selective binding of DNA molecules to magnetic beads under specific buffer conditions, followed by their separation and purification using a magnetic field – a process known as solid-phase extraction.

The protocols are phenol- and chloroform-free, ensuring safer handling while maintaining the integrity and purity of the DNA. The extracted DNA is suitable for immediate use in PCR, real-time PCR, SNP genotyping, next-generation sequencing and other downstream applications.

The kit can be used with the MagC 9600 Automated Nucleic Acid Extraction System (or similar platforms) following the step-by-step Automated Extraction Protocol provided, or with the Manual Protocol for bench-scale applications.

PLANT	A260/A280 (AVG.)	A260/A230 (AVG.)	AVG. YIELD (µg)
Wheat	2.0	2.2	4.1
Maize	1.9	2.1	1.6
Tea	2.0	2.1	5.7
Rice	2.0	2.0	1.5
Apple	1.8	1.5	1.3
Soybean	1.8	2.0	0.8
Cotton	1.9	2.1	8.2
Orange	1.8	1.5	1.1
Peanut	1.8	1.4	0.7
Tomato	1.9	1.8	1.6
Watermelon	2.0	2.1	1.2
Chilli	2.1	2.2	1.2
Aubergine	1.9	2.1	1.1
Pear	1.8	1.8	0.7
Sweet Potato	2.0	1.6	1.0
Pea	1.9	1.9	1.1
Oilseed rape	2.0	2.1	0.9
Potato	2.0	2.1	1.1

Table 1. Typical DNA Yields and Purity from Selected Plants

2. STORAGE AND SHELF LIFE

The kit is shipped at room temperature. Upon arrival, store at room temperature (15-25˚C) away from extremes of light, hot or cold.

3. SAFETY WARNINGS AND PRECAUTIONS

The contents of this kit should only be handled by trained laboratory personnel. It is advisable to wear suitable personal protective equipment (PPE) when using the product. In case of contact with skin or eyes, wash immediately with water.

Binding Buffer and Wash Buffer 1 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. Guanidine hydrochloride is irritating to eyes and skin. In case of contact with skin or eyes, rinse immediately with plenty of water and seek medical advice.

4. KIT COMPONENTS

- **Lysis Buffer [MP-1001M]** Lysis Buffer may form a precipitate upon storage. If necessary, warm to 37˚C until the precipitate has re-dissolved.
- **Binding Buffer [MP-1003M]** Important! Before first use, add isopropanol as instructed on the bottle.
- **Wash Buffer 1** (Concentrate) **[MP-1004M]** Important! Before first use, add ethanol as instructed on the bottle.
- **Wash Buffer 2** (Concentrate) **[MP-1005M]** Important! Before first use, add ethanol as instructed on the bottle.
- **Elution Buffer [MP-1006M]**
- **Mag Beads [MP-1002M]** Important! Mag Beads should be vortexed thoroughly before use. Freezing and high-speed centrifugation of Mag Beads is strictly prohibited and may cause irreversible damage.

N.B. All kit components should be stored between 15-25 ˚C.

ADDITIONAL MATERIALS TO BE SUPPLIED BY THE USER

REAGENTS

- Isopropanol
- 100% Ethanol
- Liquid Nitrogen (Optional)

CONSUMABLES

- 96 deep-well plates
- Extraction Mag Comb (Automated Protocol)
- 3-4 mm steel or zirconia beads
- Microfuge tubes (Manual Protocol)

EQUIPMENT

- MagC 9600 Automated Nucleic Acid Extraction System or similar (Automated Protocol)
- Turbo Grinder or other mechanical grinder
- Magnetic Rack (Manual Protocol)
- Thermal shaker (Manual Protocol)
- Centrifuge for 96-deep well plates
- Centrifuge for microfuge tubes (Manual Protocol)
- Multi-channel micropipette
- Single -channel micropipette

5. STEP-BY-STEP PROTOCOL FOR AUTOMATED EXTRACTION

1. Before first use of the kit reagents, prepare the buffers as instructed on the buffer bottles.

2. Take 50-100 mg of fresh plant tissue or about 30mg of dry weight tissue and place one sample per well/tube into a 2.0 mL deep-well plate, or microfuge tubes.

3. Grind the samples to a fine powder ready for extraction using one of the three methods below:

   a Add liquid nitrogen to each well to freeze the samples and grind the samples into a fine powder. **Immediately** add 400 µL **Lysis Buffer** to the resultant powder and mix thoroughly.

   b Freeze, lyophilise and grind the samples into a fine powder. **Immediately** add 400 µL **Lysis Buffer** to the resultant powder and mix thoroughly.

   c Add steel balls and 400 µL **Lysis Buffer** to each well or tube. Grind the samples using a Turbo Grinder or similar mechanical grinder.

   N.B. Transfer **Lysis Buffer** to the ground samples as quickly as possible to avoid DNA degradation after homogenisation.

4. Incubate the samples at room temperature for 15 minutes then centrifuge at 12,000 rpm for 5 minutes or 4,000 rpm for 15 minutes.

5. Transfer 150 µL of supernatant to the Binding Plate, Plate 1, containing 300 µL **Binding Buffer** (ensure Isopropanol has been added before first use) and 5 µL **Mag Beads** per well.

6. According to Table 2 below, add the specified amount of each reagent to the wells of a 96 deep-well plate (one plate for each Buffer, 6 plates in total) and place each plate in the specified position on the MagC 9600 instrument.

PLATE ID	PLATE POSITION	BUFFER AND VOLUME PER WELL
Binding Plate	1	150 µL Sample + 300 µL Binding Buffer + 5 µL Mag Beads
Wash 1 Plate 1	2	300 µL Wash Buffer 1
Wash 1 Plate 2	3	300 µL Wash Buffer 1
Wash 2 Plate 1	4	300 µL Wash Buffer 2
Wash 2 Plate 2	5	300 µL Wash Buffer 2
Elution Plate	6	100 µL Elution Buffer

Table 2. Setting up the MagC 9600: Buffer Reagents and Volumes for Plates 1-6.

7. Place the Extraction Mag Comb onto the MagC 9600 instrument and run the **Plant Genomic DNA Extraction Program** detailed in Table 3.

	STEP 1	STEP 2	STEP 3	STEP 4	STEP 5	STEP 6	STEP 7	STEP 8
Name	Load	Binding	Washing	Washing	Washing	Washing	Elution	Unload
Slot	1	1	2	3	4	5	6	4
Volume (µL)	450	450	300	300	300	300	100	300
Temp Switch	Off	Off	Off	Off	Off	Off	On	Off
Temp (℃)	-	-	-	-	-	-	65	-
Speed	-	5	8	8	8	8	10	-
Amplitude (%)	-	35	25	25	25	25	50	-
Time (sec)	-	300	60	60	60	60	300	-
Wait Model	-	-	-	-	-	-	Up	-
Time (sec)	-	0	0	0	0	0	180	-
Model	-	Advanced	Advanced	Advanced	Advanced	Advanced	Advanced	-
Speed	-	2	2	2	2	2	2	-
Loop	-	3	2	2	2	2	3	-
Time (sec)	-	60	30	30	30	30	60	-

Table 3. Plant Genomic DNA Extraction Program on the MagC 9600.

6. STEP-BY-STEP PROTOCOL FOR MANUAL EXTRACTION

1. Before first use of the kit, prepare the buffers as instructed on the buffer bottles.

2. Take 50-100 mg of fresh plant tissue or about 30mg of dry weight tissue and place one sample per well/tube into a 2.0 mL deep-well plate, or microfuge tubes.

3. Grind the sample to a fine powder ready for extraction using one of the three methods below:

   a Add liquid nitrogen to each well to freeze the samples and grind the samples into a fine powder. **Immediately** add 400 µL **Lysis Buffer** to the resultant powder and mix thoroughly.

   b Freeze, lyophilise and grind the samples into a fine powder. **Immediately** add 400 µL **Lysis Buffer** to the resultant powder and mix thoroughly.

   c Add steel balls and 400 µL **Lysis Buffer** to each well or tube. Grind the samples using a Turbo Grinder or similar mechanical grinder.

   NB. Transfer **Lysis Buffer** to the ground samples as quickly as possible to avoid DNA degradation after homogenisation.

4. Incubate the samples at room temperature for 15 minutes then centrifuge at 12,000 rpm for 5 minutes or 4,000 rpm for 15 minutes.

5. Transfer 150 µL of supernatant to a 96 deep-well plate or tube containing 300 µL **Binding Buffer** (ensure isopropanol has been added before first use) and 5 µL **Mag Beads** per well, vortex at 1,600 rpm for 5 minutes.

6. Place all tubes or plate in the magnetic rack and wait for 1 minute, then carefully remove & discard the clear supernatant from each sample taking care not to disturb the pellet.

7. Add 300 µL **Wash Buffer 1** to each tube/well (ensure 100% ethanol has been added before use), and vortex at 1,600 rpm for 1 minute. Place all tubes/plate in the magnetic rack and wait for 1 minute, carefully remove & discard the clear supernatant from each sample without disturbing the pellet.

8. Repeat step 7.

9. Add 300 µL **Wash Buffer 2** to each tube/well (ensure 100% ethanol has been added before use), and vortex at 1,600 rpm for 1 minute. Place all tubes/plate in the magnetic rack and wait for 1 minute, carefully remove & discard the clear supernatant from each sample without disturbing the pellet.

10. Repeat step 9.

11. Let the tubes/wells air dry with caps open for 5 minutes.

12. Add 100 µL **Elution Buffer** to each tube/well. Place the plate/tubes on a thermal shaker (set at 65 °C) and shake at 1,600 rpm for 5 minutes.

13. Place the plate/tubes in the magnetic rack and wait for 1 minute. Then, transfer the eluted DNA samples to a fresh tube/plate and either use immediately or store at -20˚C.

7. PLANTS WITH SECONDARY METABOLITES

Some plant species contain high levels of secondary metabolites such as polyphenols, polysaccharides, or lipids that can make successful DNA extraction and downstream applications such as PCR more challenging. For these species, we recommend trying the following protocol adjustments to improve DNA yield and purity:

1. High polyphenols (e.g., tea, grape, cotton, palm)**:**
Polyphenols can bind to nucleic acids and inhibit enzymatic reactions. To minimise their impact, add either:

- DTT to Lysis Buffer at a final concentration of 50 mM, or
- β-mercaptoethanol to the Lysis Buffer at a final concentration of 2%.

2. High polysaccharides (e.g., cacti, orchids, sweet potato, algae)**:**
Excess polysaccharides can co-precipitate with DNA and affect downstream use. To reduce contamination:

- Grind tissue in liquid nitrogen to limit mucilage release.
- Reduce tissue input to ≤20 mg per extraction.

3. High lipid content (e.g., oily seeds, avocado)**:**
Lipids can interfere with lysis and binding efficiency. To improve extraction:

- Add Proteinase K to the Lysis Buffer.
- Incubate at 55 °C for 15 minutes during lysis.

4. Complex or woody tissues (e.g., mosses, lichens, woody stems)**:**
Tough cell walls may reduce DNA release. To improve recovery:

- Extend mechanical disruption.
- Optionally add Proteinase K to the Lysis Buffer.

These simple modifications allow the kit to be used successfully with a wide range of species while maintaining high-quality DNA suitable for PCR and other downstream applications.

8. ADDITIONAL INFORMATION

- All kit components and buffers can be stored at room temperature (15-25°C). Once ethanol and isopropanol are added, Binding Buffer, Wash Buffer 1 and Wash Buffer 2 remain stable for up to 3 months when stored at room temperature.
- Yields of DNA may vary depending on sample type, age, and storage conditions. We recommend using young leaf samples, or samples containing a large number of cells.
- The lysis procedure is most effective with well homogenised samples. Recommended methods include grinding the sample into a powder using a mortar and pestle in the presence of liquid nitrogen or homogenising it with a Turbo Grinder or other mechanical grinder in the presence of steel or zirconia beads.
- To minimise DNA degradation, avoid repeated freezing and thawing of the samples and perform isolation from fresh material, or material that has been immediately frozen and stored at -80 °C.
- Clarified lysates can be stored in Lysis Buffer either overnight at room temperature or for up to 3 days at 2-8 °C before adding Binding Buffer and Mag Beads. Ensure that samples are mixed thoroughly before use.
- Volumes for reagent mixes are given per sample. We recommend that you prepare master mixes for larger sample numbers. To calculate volumes for master mixes, refer to the per-well volume and add 5-10% overage.
- The volume of Elution Buffer can be reduced to 70 µL if higher DNA concentration is needed or increased to 200 µL if lower DNA concentration is needed.
- The elution plate or tube may exhibit slight discoloration due to residual bead haze after elution. This minimal bead residue does not interfere with downstream applications.

9. TROUBLESHOOTING

Scenario 1: Low purity (A260/A280 < 1.7)

- Possible Cause: Presence of impurities due to insufficient washing.
- Solution: Reduce the amount of starting material or dilute the sample. You can also increase the number of wash steps to improve purity.

Scenario 2: Low yield

- Possible Cause: Incomplete lysis or insufficient elution.
- Solution: Ensure thorough sample disruption (e.g., more thorough mashing or mechanical homogenisation). Extend lysis time or increase the temperature of lysis to 65 °C. Ensure Elution Buffer is at 65 °C.

Scenario 3: DNA degradation

- Possible Cause: Repeated freezing/thawing of starting material.
- Solution: Perform extraction on fresh plant material or material that has been immediately frozen and stored at -80°C only. Excessive mechanical stress during grinding, or activation of endogenous nucleases.

- Possible Cause: Avoid over-grinding.
- Solution: Make sure Lysis Buffer is added immediately after grinding samples, as delays can lead to degradation.

Scenario 4: Poor downstream performance

- Possible Cause: Residual inhibitors present in the purified DNA.
- Solution: Reduce the amount of starting material to minimise inhibitor carryover. Ensure magnetic beads are completely dried and free of ethanol before elution. Use fluorescence-based quantification methods to accurately assess DNA yield and quality. Consider modifications suggested in section 7 where relevant.

10. ORDERING INFORMATION

For ordering details, please contact orders@3crbio.com.

11. SUPPORT

If you require any support with the use of Mag Plant Pro-M Genomic DNA Extraction Kit or other 3CR Bioscience products, please contact our Technical Support team at support@3crbio.com.

12. LICENCE INFORMATION

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

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