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MAG SEED PRO-S GENOMIC DNA EXTRACTION KIT USER GUIDE

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CONTENT

1. DESCRIPTION	3
2. STORAGE AND SHELF LIFE	4
3. SAFETY WARNINGS AND PRECAUTIONS	4
4. KIT COMPONENTS	4
5. STEP-BY-STEP PROTOCOL FOR AUTOMATED EXTRACTION	5
6. STEP-BY-STEP PROTOCOL FOR MANUAL EXTRACTION	7
7. ADDITIONAL INFORMATION	8
8. TROUBLESHOOTING	8
9. ORDERING INFORMATION	9
10. SUPPORT	9
11. LICENCE INFORMATION	9

1. DESCRIPTION

The Mag Seed Pro-S Genomic DNA Extraction Kit has been developed to extract genomic DNA from a wide variety of seeds, 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 supports a variety of seed sizes and types:

- **Common seeds** (e.g., rice, wheat, barley, Arabidopsis): A single seed is often sufficient for standard molecular applications.
- **Large seeds** (e.g., corn/maize, bean, coconut): Only a small chip (20–30 mg) from the endosperm or cotyledon is used, rather than the entire seed.
- **Very small seeds** (e.g., some grasses, cabbage): A single seed may not yield enough DNA, so pooling multiple seeds (typically 5–10) from the same plant or genetic line is recommended.

• SEED	A260/A280 (AVG.)	A260/A230 (AVG.)	AVG. YIELD (µg)
Wheat	2.1	2.3	1.1
Maize (embryo)	2.1	2.3	0.9
Sorghum	2.0	2.5	0.4
Rice	2.1	2.2	0.3
Soybean	2.1	2.1	1.3
Cotton (skinless)	1.9	2.0	0.8
Tomato	2.0	2.6	0.3
Watermelon (skinless)	1.9	2.6	0.5
Cabbage	2.0	2.7	0.3

Table 1. Typical DNA Yields and Purity from Selected Seeds

Removing the seed coat (e.g., watermelon, muskmelon, cotton) prior to processing is strongly recommended where possible. Seed coats can harbour polysaccharides, polyphenols, soil residues, pesticides, or microbial contaminants that co-extract with nucleic acids, which can inhibit 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.

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.

Wash Buffer 1 contains 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 [MS-2001S]** Lysis Buffer may form a precipitate upon storage. If necessary, warm to 37°C until the precipitate has re-dissolved.
- **Binding Buffer [MS-2003S]** **Important!** Before the first use, add isopropanol as instructed on the bottle.
- **Wash Buffer 1 [MS-2004S]** (concentrate) **Important!** Before the first use, add ethanol as instructed on the bottle.
- **Wash Buffer 2 [MS-2005S]** (concentrate) **Important!** Before the first use, add ethanol as instructed on the bottle.
- **Elution Buffer [MS-2006S]**
- **Mag Beads [MS-2002S]** **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.
- **Pro K (20mg/mL) [MS-2007S]** This can be stored at 15-25 °C for up to one year, and 2-8 °C for up to 18 months.

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 kit use, prepare the buffers as instructed on the buffer bottles.
2. Take 20 mg of seed and mash by physical means. Place one sample per well/tube into a 2.0 mL deep-well plate or microfuge tubes.
3. Add steel balls, 10 μ L **Pro K** and 600 μ L **Lysis Buffer** to each well or tube. Grind the samples using the Turbo Grinder or other mechanical grinder.
4. Incubate the samples at room temperature for 15 minutes. Next, centrifuge at 12,000 rpm for 5 minutes or 4,000 rpm for 15 minutes.
5. Transfer 100 μ L of supernatant to the Binding Plate, Plate 1, containing 200 μ L **Binding Buffer** (ensure Isopropanol has been added before first use) and 3.3 μ L **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 AND POSITION NUMBER	BUFFER AND VOLUME PER WELL
Binding Plate	1	100 μ L Sample + 200 μ L Binding Buffer + 3.3 μ L Mag Beads
Wash 1 Plate 1	2	200 μ L Wash Buffer 1
Wash 1 Plate 2	3	200 μ L Wash Buffer 1
Wash 2 Plate 1	4	200 μ L Wash Buffer 2
Wash 2 Plate 2	5	200 μ L Wash Buffer 2
Elution Plate	6	100 μ L 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 and run the **Seed 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)	300	300	200	200	200	200	100	200
Temp Switch	Off	Off	Off	Off	Off	Off	On	Off
Temp ($^{\circ}$C)	-	-	-	-	-	-	65	-
Speed (Hz)	-	5	8	8	8	8	10	-
Amplitude (%)	-	25	15	15	15	15	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 (Hz)	-	2	2	2	2	2	2	-
Loop	-	3	2	2	2	2	3	-
Time (sec)	-	60	30	30	30	30	60	-

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

6. STEP-BY-STEP PROTOCOL FOR MANUAL EXTRACTION

1. Before first kit use, prepare the buffers for use as instructed on the buffer bottles.
2. Take 10-20 mg of seed and mash by physical means. Place one sample per well/tube into a 2.0 mL deep-well plate or microfuge tubes.
3. Add steel balls, 10 μ L of **Pro K** and 600 μ L **Lysis Buffer** to each well. Grind the samples using a Turbo Grinder or similar mechanical grinder.
4. Incubate the samples at room temperature for 15 minutes. Next, centrifuge at 12,000 rpm for 5 or 4,000 rpm for 15 minutes.
5. Transfer 100 μ L of supernatant to a 96 deep-well plate or tube containing 200 μ L **Binding Buffer** (ensure isopropanol has been added before first use) and 3.3 μ L **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 200 μ L **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 200 μ L **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 μ L **Elution Buffer** to each tube/well. Place the plate/tubes on a thermal shaker (set at 65 °C) and shake at 1600 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. 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.
- Perform all steps at room temperature (15–25 °C) unless otherwise noted.
- 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.
- Differences in nucleic acid content between the embryo and endosperm are common. The embryo typically exhibits a higher concentration of nucleic acids.
- Removing the seed coat (e.g., watermelon, muskmelon, cotton) prior to processing is strongly recommended. Seed coats often harbour polysaccharides, polyphenols, soil residues, pesticides, or microbial contaminants that co-extract with nucleic acids, which can inhibit downstream applications.

8. 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. Increase the number of wash steps to improve purity.

Scenario 2: Low yield

- Possible Cause: Incomplete lysis or use of unsuitable seed tissue.
- Solution 1: Ensure thorough sample disruption (e.g., more effective mashing or mechanical homogenization). Use seed embryos when possible, as they often contain more DNA than the
- Solution 2: For the lysis step (Step 4) incubate samples for one hour at 55 °C instead of 15 mins at room temperature.

Scenario 3: DNA degradation

- Possible Cause: Excessive mechanical stress during grinding, or activation of endogenous nucleases.
- Solution: Avoid over-grinding. Immediately add lysis buffer after disrupting the 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 (e.g., Qubit) to accurately assess DNA yield and quality.

9. ORDERING INFORMATION

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

10. SUPPORT

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

11. LICENCE INFORMATION

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