MagMAX™ Plant DNA Kit

USER GUIDE

High-throughput purification of PCR-ready DNA from plant tissues

Catalog Numbers A32549 and A32580

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Revision A.0

ThermoFisher SCIENTIFIC

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Revision history: Pub. No. MAN0015954

Revision	Date	Description
A.0	11 August 2016	New document

Contents

Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The MagMAX™ Plant DNA Kit is designed for rapid, high-throughput purification of high-quality robust yields of DNA from a wide variety of plant species. The kit uses MagMAX™ magnetic beads technology, ensuring reproducible recovery of DNA that is ready for downstream PCR, sequencing, or other applications.

The procedures of the MagMAX™ Plant DNA Kit describe the isolation of DNA from 10–100 mg of plant sample, optimized for use with the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head (96DW; 96-well deep well setting), the KingFisher™ Duo Prime Magnetic Particle Processor (12-well deep well setting), or for manual sample processing. DNA yields vary between different species and tissues and depend on genome size, ploidy, cell number, age of tissue sample, and storage conditions (see Table 1).

Table 1 Typical DNA yields from 50 mg (leaves) or 10 mg (seeds) of various plants

Source	DNA yield
Arabidopsis thaliana leaves	up to 2 µg
Barley leaves	up to 8 µg
Bell pepper leaves	up to 5.5 µg
Canola/rapeseed leaves	up to 4 µg
Canola/rapeseed seeds	up to 1.5 µg
Corn seeds	up to 1 µg
Corn leaves	up to 5 µg
Cotton leaves	up to 9.5 µg
Cotton seeds	up to 5 µg
Dill/soya leaves	up to 6.5 µg
Green onion leaves	up to 5 µg
Oat seeds	up to 5 µg
Oat leaves	up to 8 µg

Source	DNA yield
Pine needles	up to 3 µg
Spinach leaves	up to 3 µg
Tomato leaves	up to 3 µg
Wheat leaves	up to 10 µg
Wheatgrass leaves	up to 15 µg

Contents and storage

Table 2 MagMAX™ Plant DNA Kit (Cat. Nos. A32549 and A32580)

Contents	Cat. No. A32549 (96 reactions)	Cat. No. A32580 (384 reactions)	Storage
Lysis Buffer A	60 mL	4 × 60 mL	15 to 30°C
Lysis Buffer B	8.4 mL	4 × 8.4 mL	
RNase A	2 × 1.4 mL	8 × 1.4 mL	–25°C to –15°C
Precipitation Solution	16.5 mL	4 × 16.5 mL	15 to 30°C
DNA Binding Beads	2 × 1.4 mL	8 × 1.4 mL	2 to 8°C
Wash Solution 1 Concentrate[1]	45 mL	4 × 45 mL	15 to 30°C
Wash Solution 2 Concentrate[1]	60 mL	4 × 60 mL	
Elution Buffer	30 mL	4 × 30 mL	

[1] See "Before first use of the kit" on page 11.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Table 3 Materials required for DNA purification (all methods)

Item	Source
Equipment	
Heat block, water bath, or thermomixer at 65°C	MLS
Centrifuge capable of ≥16,000 × *g* for microcentrifuge tubes	MLS
Centrifuge capable of 3000–4000 × *g* with swinging-bucket rotor for 96-well deep well plates	MLS

Item	Source
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Vortex mixer, or equivalent	MLS
Tubes, plates, and other consumables	
Nonstick, RNase-free Microfuge Tubes (1.5 mL)	AM12450
(For purification in plate format) MicroAmp™ Clear Adhesive Film	4306311
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
Disposable gloves	MLS
Reagents	
(For purification from seeds) Dithiothreitol (DTT)	MLS
(For purification from woody, lignified, and polyphenol-rich samples) Polyvinylpyrrolidone (PVP)	MLS
Ethanol, 96–100% (molecular biology grade)	MLS

Table 4 Additional materials required for manual disruption

Item	Source
Equipment	
Mortar and pestle	MLS
Spatula	MLS
Reagents	
Liquid nitrogen	MLS

Table 5 Additional materials required for automated disruption

Item	Source
Equipment	
Homogenizer, one of the following:	
Fisher Scientific™ Bead Mill 24 Homogenizer, or equivalent	Fisher Scientific 15-340-163
Fisher Scientific™ Bead Mill 4 Homogenizer, or equivalent	Fisher Scientific 15-340-164

Item	Source
Fisher Scientific™ PowerGen™ High-Throughput Homogenizer, or equivalent	Fisher Scientific 02-215-503
3-mm steel or tungsten carbide beads	MLS
Plates or tubes	
Plates or tubes recommended by the homogenizer's manufacturer	MLS

Table 6 Additional materials required for automated purification

Item	Source
Magnetic particle processor, one of the following:	
KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
Plates and combs	
96 deep-well plates, one of the following:	
MagMAX™ Express-96 Deep Well Plates	4388476
KingFisher™ Deepwell 96 Plate	95040450
96-well standard plates, one of the following:	
MagMAX™ Express-96 Standard Plates	4388475
KingFisher™ 96 KF microplate (200 µL)	97002540
Tip comb, compatible with the magnetic particle processor used:	
KingFisher™ 96 Tip Comb for DW Magnets	97002534
MagMAX™ Express-96 Deep Well Tip Combs[1]	4388487
KingFisher™ Duo 12-Tip Comb, for Microtiter 96 Deepwell plate	97003500
Other consumables	
(For KingFisher™ Duo Prime Magnetic Particle Processor) KingFisher™ Duo Elution Strip	97003520

[1] Compatible with the KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head.

Table 7 Additional materials required for manual purification

Item	Source
Equipment	
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific 11-676-337
Thermomixer with associated block (1.5 mL, 2 mL, or 96-well plate)	MLS
(For manual purification in tube format) Vortex Adapter-60	AM10014
Plates and accessories	
(For manual purification in plate format) ABgene Storage Plate, 96-well, 1.2 mL, square well, U-bottomed	AB1127
Magnetic stand, compatible with selected manual purification format:	
(For manual purification in plate format) Magnetic Stand-96	AM10027
(For manual purification in tube format) DynaMag™–2 magnet	12321D

If needed, download the KingFisher™ Flex or Duo Prime program

The programs required for this protocol are not pre-installed on the KingFisher™ Flex Magnetic Particle Processor 96DW or on the KingFisher™ Duo Prime Magnetic Particle Processor.

1. On the MagMAX™ Plant DNA Kit product web page, scroll down to the **Product Literature** section.
2. Right-click on the appropriate program file to download the program to your computer:
   - **KingFisher™ Flex Magnetic Particle Processor 96DW: A32549_Plant_DNA_Flex**
   - **KingFisher™ Duo Prime Magnetic Particle Processor: A32549_Plant_DNA_Duo**
3. Select **Save as Target** to download to your computer.
4. Refer to the manufacturer's documentation for instructions for installing the program on the instrument.

Methods

Procedural guidelines

General procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Use disposable, individually wrapped, sterile plasticware.
- Use only sterile, new pipette tips and microcentrifuge tubes.
- Always change pipette tips between liquid transfers to avoid cross-contamination. We recommend the use of aerosol-resistant pipette tips.
- If you use a titer plate shaker other than the Thermo Scientific™ Compact Digital Microplate Shaker, ensure that the plate fits securely on your shaker and test speeds using your specific set-up and volumes. Ideal speeds should allow for vigorous mixing without splashing.
- 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.
- After addition of Lysis Buffer B to Lysis Buffer A, a white precipitate may be observed. It does not affect yield, purity, or downstream applications.
- Clarified lysates can be stored in Lysis Buffer mix overnight at 2–8°C or at –20°C for up to 7 days before adding the ethanol and the DNA Binding Beads. Ensure that samples are completely thawed and mixed thoroughly before use.

Guidelines for sample handling and homogenization

- We recommend using 10–100 mg of fresh plant sample or up to 20 mg of dried plant sample as starting material for manual disruption and 10–50 mg of fresh plant sample or up to 10 mg of dried plant sample as starting material for automated disruption.
- Yields of DNA may vary depending on sample type, age, and storage conditions. We recommend using young leaf samples and if possible, keeping the plant in the dark for about 12 hours before collecting samples. This step reduces the polysaccharide and polyphenolic content within the sample. Appropriate sample storage is essential for reproducibility and maximum DNA yields.
- The lysis procedure is most effective with well homogenized samples. Appropriate methods include grinding into a powder with a mortar and pestle in the presence of liquid nitrogen or homogenizing with a bead mill or high-throughput homogenizer in the presence of steel or tungsten carbide beads.

Before you begin

Before first use of the kit

- Prepare the Wash Solutions from the concentrates:
  - Add 135 mL of 96–100% ethanol to Wash Solution 1 Concentrate, mix, check the box on the bottle label to indicate that this step has been completed, then store at room temperature.
  - Add 180 mL of 96–100% ethanol to Wash Solution 2 Concentrate, mix, check the box on the bottle label to indicate that this step has been completed, then store at room temperature.

Before each use of the kit

- Check all the solutions in the kit for salt precipitation. Dissolve any precipitates by warming the solution at 37°C, then equilibrate to room temperature.
- Resuspend the DNA Binding Beads well by vortexing.
- Set heat block, water bath, or thermomixer to 65°C.
- **For manual purification only:** Set up an extra thermomixer to 70°C (for eluting the DNA).
- Supplement Lysis Buffer A if needed.

  Note: Supplement only the volume of Lysis Buffer A that is needed for immediate isolation. We recommend using the supplemented Lysis Buffer A only during the day that it is prepared.

  - **For woody, lignified, or polyphenol-rich samples (branches, twigs, needles, and wax-coated leaves):** Add PVP to Lysis Buffer A at a 2% (w/v) final concentration.

    Note: To maximize the effectiveness of PVP, we recommend adding the PVP from a stock solution (for example, 20% w/v) instead of dissolving the powder directly into Lysis Buffer A.
  - **For seeds:** Add DTT to Lysis Buffer A at a 40-mM final concentration.

Disrupt the tissue

- To disrupt the tissue manually, proceed to "Disrupt the sample manually" on page 11.
- To disrupt the tissue using a bead mill or high-throughput homogenizer, proceed to "Disrupt the tissue using a bead mill or high-throughput homogenizer" on page 13.

Disrupt the sample manually

We recommend using 10–100 mg of fresh plant sample or up to 20 mg of dried plant sample.

1. Add liquid nitrogen to a clean mortar.
2. Freeze the plant tissue by placing it in the liquid nitrogen in the mortar.

3. Grind the tissue thoroughly using a clean pestle, then allow the liquid nitrogen to evaporate.

   Ground tissue can be used immediately or stored at –80°C until use.

4. Add 350 µL of Lysis Buffer A to a 1.5-mL microcentrifuge tube.

5. Transfer up to 100 mg of sample into the tube containing Lysis Buffer A, then mix thoroughly by vigorously vortexing for 10–20 seconds.

   IMPORTANT! Transfer the ground tissue to the Lysis Buffer A as quickly as possible to avoid DNA degradation.

6. Centrifuge briefly to collect the liquid at the bottom of the tube.

7. Add 50 µL of Lysis Buffer B and 20 µL of RNase A, then mix thoroughly by vigorously vortexing for 10–20 seconds.

8. Incubate at 65°C for 10 minutes, vigorously vortexing occasionally.

   Alternatively, you can use a vigorously shaking thermomixer set at 65°C.

9. Centrifuge briefly to collect the liquid at the bottom of the tube.

10. Add 130 µL of Precipitation Solution, then mix by inverting the tube 2–3 times.

11. Incubate for 5 minutes on ice.

12. Centrifuge for 5 minutes at maximum speed (≥ 16,000 × *g*) to clear the plant lysate.

13. Transfer 400 µL of the supernatant to a clean 1.5-mL microcentrifuge tube (manual purification) or to the well of a 96-well plate (manual or automated purification).

STOPPING POINT You can store the clarified lysates as indicated in "General procedural guidelines" on page 10.

Proceed with the DNA purification.

- For automated purification using KingFisher™ Flex Magnetic Particle Processor 96DW, proceed to "Purify DNA using KingFisher™ Flex Magnetic Particle Processor 96DW" on page 14.
- For automated purification using KingFisher™ Duo Prime Magnetic Particle Processor, proceed to "Purify DNA using KingFisher™ Duo Prime Magnetic Particle Processor" on page 15.
- For manual purification, proceed to "Purify DNA manually" on page 16.

Disrupt the tissue using a bead mill or high-throughput homogenizer

Plant tissue can be homogenized with bead mill or high-throughput homogenizers. We recommend using these homogenizers paired with steel or tungsten carbide beads. High-throughput homogenizers offer an appropriate method for handling 96 samples simultaneously. For low-throughput homogenization, it is possible to use 1.5– or 2.0–mL microcentrifuge tubes.

We recommend using 10–50 mg of fresh plant sample or up to 10 mg of dried plant sample.

1. Place the plant tissue in a 96-well plate or a microcentrifuge tube according to the manufacturer recommendations.
2. Add 500 µL of Lysis Buffer A, 70 µL of Lysis Buffer B, and 20 µL of RNase A, then mix thoroughly by briefly vortexing.
3. Grind the sample according to the manufacturer recommendations.
4. After sample homogenization, incubate the sample at 65°C for 10 minutes, vigorously vortexing occasionally.

   Alternatively, you can use a vigorously shaking thermomixer set at 65°C.
5. Add 130 µL of Precipitation Solution, then mix by inverting the sample 2–3 times.
6. Incubate for 5 minutes on ice.
7. Centrifuge for 10 minutes at maximum speed (≥ 16,000 × *g* for microcentrifuge tubes or 3000–4000 × *g* for plates) to clear the plant lysate.
8. Transfer 400 µL of the supernatant to a clean 1.5-mL microcentrifuge tube (manual purification) or to the well of a 96-well plate (manual or automated purification).

STOPPING POINT You can store the clarified lysates as indicated in "General procedural guidelines" on page 10.

Proceed with the DNA purification.

- For automated purification using KingFisher™ Flex Magnetic Particle Processor 96DW, proceed to "Purify DNA using KingFisher™ Flex Magnetic Particle Processor 96DW" on page 14.
- For automated purification using KingFisher™ Duo Prime Magnetic Particle Processor, proceed to "Purify DNA using KingFisher™ Duo Prime Magnetic Particle Processor" on page 15.
- For manual purification, proceed to "Purify DNA manually" on page 16.

Purify DNA using KingFisher™ Flex Magnetic Particle Processor 96DW

Set up the processing plates

Ensure that ethanol has been added to the Wash Solution Concentrates (see "Before first use of the kit" on page 11).

Add processing reagents to the wells of 96-well plates as indicated in the following table.

Table 8 Processing plates setup

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Sample Plate[2]	1	Deep Well	Plant lysate	400 µL
			DNA Binding Beads[3]	25 µL
			96–100% Ethanol	400 µL
Wash 1 Plate	2	Deep Well	Wash Solution 1	800 µL
Wash 2 Plate_1	3	Deep Well	Wash Solution 2	800 µL
Wash 2 Plate_2	4	Deep Well	Wash Solution 2	800 µL
Elution Plate	5	Standard	Elution Buffer	150 µL
Tip Plate	6	Place a KingFisher™ 96 Tip Comb for DW Magnets in a Standard Plate.		
	7	Empty		
	8	Empty		

[1] Position on the instrument.

[2] Prepare the Sample Plate last. Add reagents in the order listed in the table.

[3] Resuspend well by vortexing before use.

Bind, wash, and elute the DNA

1. Ensure that the instrument is set up for processing with the deep-well magnetic head and has the standard 96-well heating block installed.
2. Select the **A32549_Plant_DNA_Flex** program on the instrument.
3. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 8).

4. At the end of the run (approximately 39 minutes after initial start), remove the Elution Plate immediately from the instrument and transfer the eluate to a new tube or plate.

   If using a plate, seal the wells immediately with an adhesive film after having transferred the eluate.

   Note: The plate may be discolored from a remaining bead haze. This minimal bead residue does not interfere with downstream applications.

The purified DNA is ready for immediate use. Alternatively, store the purified DNA:

- At 2–8°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Purify DNA using KingFisher™ Duo Prime Magnetic Particle Processor

Set up the processing plate and the Elution Strip

Ensure that ethanol has been added to the Wash Solution Concentrates (see "Before first use of the kit" on page 11).

1. Add processing reagents to the wells of a 96-well plate as indicated in the following table.

Table 9 Processing plate setup

Row ID	Plate row	Reagent	Volume per well
Sample[1]	A	Plant lysate	400 µL
		DNA Binding Beads[2]	25 µL
		96–100% Ethanol	400 µL
Tip Comb	B	Place a KingFisher™ Duo 12-Tip Comb in Row B.	
	C	Empty	
	D	Empty	
Wash 1	E	Wash Solution 1	800 µL
Wash 2_1	F	Wash Solution 2	800 µL
Wash 2_2	G	Wash Solution 2	800 µL
	H	Empty	

[1] Prepare the Sample Row last. Add reagents in the order listed in the table.

[2] Resuspend well by vortexing before use.

2. Prepare the Elution Strip as indicated in the following table.

Table 10 Elution Strip setup

Consumable	Reagent	Volume per well
KingFisher™ Duo Elution Strip	Elution Buffer	100 µL

Bind, wash, and elute the DNA

1. Ensure that the instrument is set up for processing with the 12–pin magnetic head and has the 12–well heating block installed.
2. Select the **A32549_Plant_DNA_Duo** program on the instrument.
3. Start the run and load the prepared processing plate and Elution Strip in their positions when prompted by the instrument (see Table 9 and Table 10).
4. At the end of the run (approximately 35 minutes after initial start), remove the Elution Strip immediately from the instrument and transfer the eluate to a new tube or plate.

   If using a plate, seal the wells immediately with an adhesive film after having transferred the eluate.

   Note: The Elution Strip may be discolored from a remaining bead haze. This minimal bead residue does not interfere with downstream applications.

The purified DNA is ready for immediate use. Alternatively, store the purified DNA:

- At 2–8°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Purify DNA manually

Manual purification can be performed either in 1.5–mL microcentrifuge tubes or in 1.2–mL U-bottomed 96–well plates.

Bind the DNA to the beads

1. Add 25 µL of DNA Binding Beads to 400 µL of the plant lysate.

   Note: Resuspend DNA Binding Beads well by vortexing before use.
2. Add 400 µL of 96–100% ethanol, then mix thoroughly according to the following table to obtain a uniform suspension.

Format	Mixing
Tube	• Vigorously vortex in a vortex adapter for 5 minutes. • Centrifuge briefly to collect the liquid at the bottom of the tube.
Plate	Ensure that mixture is homogeneous by gently pipetting up and down 5 times, then place on a microplate shaker for 5 minutes at 500 rpm.

3. Place the sample on the magnetic stand according to the following table.

Format	Bead collecting time
Tube	2 minutes or until the solution clears and the beads are collected against the magnet.
Plate	5 minutes or until the solution clears and the beads are collected against the magnet.

4. Without removing the sample from the magnetic stand, carefully pipette and discard the supernatant.

   Ensure that you remove all the supernatant.

5. Remove the sample from the magnetic stand.

Wash the DNA on the beads

Ensure that ethanol has been added to the Wash Solution Concentrates (see "Before first use of the kit" on page 11).

1. Add 400 µL of Wash Solution 1 to the beads, then mix thoroughly according to the following table to obtain a uniform suspension.

Format	Mixing
Tube	• Vigorously vortex in a vortex adapter for 1 minute. • Centrifuge briefly to collect the liquid at the bottom of the tube.
Plate	Vigorously mix on a microplate shaker for 1 minute at 750 rpm.

   Note: Beads may clump together, making a uniform suspension difficult to obtain. However, it has no effect on the final yield.

2. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are collected against the magnet.

3. Without removing the sample from the magnetic stand, carefully pipet and discard the supernatant.

   Ensure that you remove all the supernatant.

4. Remove the sample from the magnetic stand.

5. Repeat step 1-step 4 twice with 400 µL of **Wash Solution 2**.

6. Incubate the sample for 5 minutes on the magnetic stand to eliminate the remaining ethanol.

   - **Tube format:** Incubate with the lid open.
   - **Plate format:** Incubate with the plate uncovered.

Elute the DNA

1. Add 150 µL of Elution Buffer to the beads.

   Note: The volume of Elution Buffer can be reduced to 50 µL if higher DNA concentration is needed or increased to 200 µL if lower DNA concentration is needed.

2. Mix thoroughly according to the following table to obtain a uniform suspension.

Format	Mixing
Tube	• Vigorously vortex for 20 seconds. • Centrifuge briefly to collect the liquid at the bottom of the tube.
Plate	Vigorously mix on a microplate shaker for 20 seconds at 700 rpm.

3. Incubate the sample in a thermomixer at 70°C for 5 minutes at 600–700 rpm.

   Note: If you are processing the sample using a plate format, cover the plate before incubation with an adhesive film.

4. *(Tube format only)* Centrifuge briefly to collect the liquid at the bottom of the tube.

5. Place the sample on the magnetic stand for 5 minutes or until the solution clears and the beads are collected against the magnet.

6. Without removing the sample from the magnetic stand, carefully transfer the DNA-containing supernatant to a clean tube or plate.

   If using a plate, seal the wells immediately with an adhesive film after having transferred the eluate.

The purified DNA is ready for immediate use. Alternatively, store the purified DNA:

- At 2–8°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

A

Troubleshooting

Observation	Possible cause	Recommended action
The DNA yield is low	An excess of sample was used during the lysate preparation.	Reduce the amount of starting material. Do not use more plant tissue than indicated.
	The sample was not sufficiently homogenized.	To disrupt adequately the cell wall and therefore release the DNA, it is important to homogenize the sample thoroughly. Efficient homogenization and lysis of the plant cells increases the DNA yield.
	96–100% ethanol was not added to the Wash Solution Concentrates.	Ensure that ethanol was added to Wash Solution 1 and Wash Solution 2 before use as indicated in "Before first use of the kit" on page 11.
	The sample was not stored properly.	Use fresher samples. Improper storage of the samples may compromise the yield.
	The elution volume or temperature is not correct.	Ensure you use sufficient Elution Buffer to cover completely the magnetic beads during the elution step.
		Heat the samples to 70°C during the elution step.
		Always preheat the heat source to 70°C before performing the incubation.
The DNA purity is low	The sample was not stored properly.	Use fresher samples. Improper storage of the samples may compromise the purity.
	Salt carryover occurred.	Remove the supernatant completely after the binding step and each washing step.
The DNA is degraded	The sample was frozen and thawed repeatedly.	Avoid repeated freeze/thaw cycles of the samples.
		Use a new sample for DNA isolation.
		Perform DNA isolation from fresh samples when possible.
	The sample material was not properly mixed with Lysis Buffer A.	Ensure that all ground materials are thoroughly mixed with Lysis Buffer A. Degradation can occur in particles that are left to dry on the walls on the tube.
	(Automated disruption only) The sample was homogenized for too long or at too high of a speed.	Decrease the time or the speed of the sample homogenization.

Observation	Possible cause	Recommended action
The DNA is contaminated with RNA	The sample was not treated with RNase A.	Ensure that you treat the sample with RNase A as describe in the isolation protocols.
Magnetic beads are present in the eluate	Loose beads were inadvertently transferred with the eluate.	Avoid carrying over magnetic beads when transferring the DNA-containing supernatant to a new tube.
		Centrifuge the eluate at full speed for 1 minute, then transfer the supernatant to a new tube. Alternatively, recapture the magnetic beads on the magnetic stand, then transfer the eluate to a new tube or plate.
Downstream enzymatic reactions are inhibited	Salt carryover occurred.	Before the elution step, ensure that the magnetic beads are first washed with Wash Solution 1, then twice with Wash Solution 2.
		Remove the supernatant completely after the binding step and each washing step.
	Inhibitors are present in the sample.	Dilute the DNA sample 10–100 times before the PCR.
		Use enzymes that are designed and optimized to be effective in harsh environments, and work in presence of inhibitors.
		Add Bovine Serum Albumen (BSA) to the PCR reactions.
		Ensure that fresh preparations of DTT or PVP are added to Lysis Buffer A if your sample requires it (see "Before each use of the kit" on page 11).
		Some seeds do benefit from addition of PVP to Lysis Buffer A. If DTT supplementation to Lysis Buffer A was already attempted, try again with PVP instead. (see "Before each use of the kit" on page 11).

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Customer and technical support

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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