PRODUCT INFORMATION

Thermo Scientific
MagJET Plant Genomic DNA Kit
#K2761, #K2762

Read Storage information (p. 4) upon receipt and store kit components appropriately!

www.thermoscientific.com/onebio

#K2761, #K2762
Lot 00000000
Exp. 00.0000

CERTIFICATE OF ANALYSIS

Thermo Scientific™ MagJET™ Plant Genomic DNA Kit is qualified by isolating genomic DNA from 100 mg of plant tissue following the protocols outlined in the manual. The quality of isolated DNA is evaluated spectrophotometrically and by agarose gel electrophoresis. The purified DNA has an A_{260}/A_{280} ratio of 1.8±0.3. A single band of more than 30 kb is observed after agarose gel electrophoresis and ethidium bromide staining. The functional quality of purified DNA is evaluated by digestion with restriction endonucleases.

Quality authorized by: **Jurgita Žilinskienė**

Rev.1 53

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COMPONENTS OF THE KIT

MagJET Plant Genomic DNA Kit	#K2761 96 preps	#K2762 384 preps
RNase A	2 × 1.4 mL	7 × 1.2 mL
Lysis Buffer A for MagJET Plant gDNA Kit	60 mL	225 mL
Lysis Buffer B for MagJET Plant gDNA Kit	8.4 mL	32 mL
Precipitation Solution for MagJET Plant gDNA Kit	16.5 mL	60 mL
MagJET Magnetic Beads	2 × 1.4 mL	10.6 mL
Wash Buffer 1 (conc.) for MagJET Plant gDNA Kit	45 mL	2 × 45 mL
Wash Buffer 2 (conc.) for MagJET Plant gDNA Kit	60 mL	3 × 60 mL
Elution Buffer	30 mL	70 mL

STORAGE

RNase A solution is stable at room temperature as long as the vial remains sealed. After being opened it should be stored at -20°C. MagJET Magnetic Beads should be stored at 4°C. Other components of the kit should be stored at room temperature (15-25°C).

DESCRIPTION

The MagJET Plant Genomic DNA Kit is designed for automated high throughput or manual purification of genomic DNA from a wide variety of plant species. The kit exploits an efficient magnetic particle technology eliminating the need for phenol/chloroform extraction or alcohol precipitation.

The procedures of the MagJET Plant Genomic DNA Kit have been optimized for the isolation of genomic DNA from 10-100 mg plant sample. DNA yields vary between different species and tissues depending on genome size, ploidy, cell number, and age of tissue sample.

The kit utilizes paramagnetic bead technology enabling high yields and robust performance. High binding capacity, uniform particle size, and rapid magnetic response of MagJET magnetic beads makes the technology ideal for high throughput automatic nucleic acid purification, as well as for manual purification for low sample throughput.

The resulting high quality DNA is free of proteins, nucleases and other contaminants or inhibitors, and can be used in a wide range of downstream applications such as PCR, qPCR or other enzymatic reactions. See Table 1 for typical genomic DNA yields from various sources.

PRINCIPLE

The MagJET Plant Genomic DNA Kit uses the highly efficient MagJET magnetic particle-based technology for nucleic acid purification. The nucleic acid isolation process combines the simple steps of sample lysis, binding DNA to the MagJET Magnetic Beads, washing and elution. Purification protocols optimized for automated KingFisher instruments utilize a high throughput magnetic bead transfer technique where magnetic beads are transferred through different reagent plates containing lysis, binding, washing and elution reagents. This enables high throughput nucleic acid purification and eliminates multiple pipetting steps.

Alternatively, a protocol is provided where buffers and other reagents are transferred in each of the protocol steps, while magnetic beads remain captured on the wall of the tube using a magnetic rack. This allows the kit to be used for various throughput applications using a magnetic rack and manual or automated pipetting equipment.

Table 1. Typical genomic DNA yields from 50 mg of various sources.

Source	DNA yield (µg)
Arabidobsis thaliana	up to 2
Nicotiana tabacum	up to 4
Wheat	up to 15
Rape leaves	up to 4
Barley seedlings	up to 7
Corn leaves	up to 3
Rice seedlings	up to 5
Spinach	up to 3
Potato stems	up to 1.5
Soya leaves	up to 3
Green onions	up to 4
Tomato	up to 1.0

IMPORTANT NOTES

- Add the indicated volumes of ethanol (96-100%) to concentrated **Wash Buffer 1 and 2** prior to first use:

	#K2761 (96 preps) and #K2762 (384 preps)	
	Wash Buffer 1	**Wash Buffer 2**
Concentrated buffer	45 mL	60 mL
Ethanol (96-100%)	**135 mL**	**180 mL**
Total volume:	180 mL	240 mL

After preparing each solution, mark the bottle to indicate that this step has been completed.

- Check all solutions in the kit for salt precipitation before each use. Re-dissolve any precipitates by warming the solution at 37°C, and then equilibrate to room temperature (20 ±5°C).
- Wear gloves when handling **Wash Buffer 1** as this reagent contains irritants (see page 13 for SAFETY INFORMATION).
- Always change pipette tips between liquid transfers to avoid cross-contamination; we recommend the use of aerosol-barrier pipette tips.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and pipette tips.
- 8- or 12-channel pipettes and pipette tips.
- 1.5-2.0 mL microtubes.
- Thermomixer or water bath (capable of 65°C).
- Dithiothreitol (DTT) (for gDNA purification from seeds).
- Polyvinylpyrrolidone (PVP) (for gDNA purification from woody, lignified and polyphenol-rich samples).
- Disposable gloves.
- 96-100% ethanol, molecular biology grade.
- Equipment for mechanical plant tissue disruption (commercial homogenizer and steel or tungsten carbide beads).
- Mortar and pestle (for manual tissue disruption in liquid nitrogen).

- Liquid nitrogen.
- Vortex.
- Centrifuge capable of ≥ 16,000 × g for microtubes.
- Centrifuge capable of 3,000-4,000 × g with swinging – bucket rotor for 96-well plates.
- Automatic magnetic particle processor and consumables.
- Magnetic particle processing rack for the manual protocol.

BEFORE STARTING

- When using the MagJET Plant Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Set thermomixer or water bath to 65°C.

STARTING MATERIAL HANDLING AND SAMPLE HOMOGENIZATION

- Yields of DNA may vary depending on sample age, type and storage conditions. We recommend using young plant samples and if possible keep plant samples in the dark for about 12 h before collecting samples. This step reduces the polysaccharide and polyphenolic content, which may interfere in downstream applications. Appropriate sample storage is essential for reproducibility and high DNA yields.
- The lysis procedure is most effective with well homogenized, powdered samples. Suitable methods include grinding with mortar and pestle in the presence of liquid nitrogen or using steel or tungsten carbide beads. We recommend the use of commercial homogenizers or bead mills.

Manual sample disruption

1. Collect a fresh plant sample in a mortar and freeze in liquid nitrogen .
   - Recommended quantity for fresh plant sample is 10-100 mg. For dried plant sample material, use up to 20 mg.
2. Grind the plant tissue using a clean pestle. Allow the liquid nitrogen to evaporate.
   - Ground tissue can be used immediately in the DNA isolation protocol or stored at -70°C until use.
3. Immediately transfer up to 100 mg of plant tissue into a 1.5 mL microcentrifuge tube (not provided) containing 350 µL of **Lysis Buffer A**. Vortex for 10-20 s to mix thoroughly.
   - Transfer the ground tissue to the Lysis Buffer A as quickly as possible to avoid DNA degradation. All ground material must be thoroughly mixed with the Lysis Buffer A. DNA degradation can occur in particles that are left to dry on the walls of the tube.
4. Add 50 µL of **Lysis Buffer B** and 20 µL of **RNase A**. Vortex for 10-20 s to mix thoroughly.
5. Incubate at 65°C for 10 minutes, vortexing occasionally or use a shaking thermomixer.
6. Add 130 µL of **Precipitation Solution** and mix by inverting the tube 2-3 times. Incubate 5 minutes on ice.
7. To clear the plant lysate, centrifuge the samples for 5 minutes at maximum speed (≥ 20,000 × g).
8. Transfer the 400 µL of supernatant to a clean microtube or microtiter deep well 96 plate if multiple samples are going to be processed, making sure not to disturb the pellet or transfer any debris. Immediately proceed with the genomic DNA isolation protocols **A-C**.

Instructions for lyses of different plant samples.
To purify genomic DNA from woody, lignified and or polyphenol-rich samples such as branches, twigs, needles, wax-coated leaves (such as laurel) and wheat flour, supplement **Lysis Buffer A** with polyvinylpyrrolidone (PVP) at a 2% (w/v) final concentration.
To purify genomic DNA from seeds (such as *Brassica napus*), supplement **Lysis Buffer A** with dithiothreitol (DTT) at a 40 mM final concentration.

Automatic tissue disruption

Plant tissue can be homogenized with commercial homogenizers or bead mills. High-throughput homogenizers offer a suitable method for handling 96 samples simultaneously. Commercial homogenizers are usually used together with steel or tungsten carbide beads. For low throughput homogenization it is possible to use 1.5-2.0 mL microtubes for mechanical plant tissue homogenization.

1. Place ≤ 50 mg fresh plant tissue in a 96–well plate* (or 1.5-2.0 mL microtubes) in the presence of grinding beads.
   - For dried plant material, use up to 10 mg.
2. Add 500 µL of **Lysis Buffer A,** 70 µL of **Lysis Buffer B** and 20 µL of **RNase A**. Vortex for 10-20 s to mix thoroughly and grind the sample according to homogenizer instructions.
3. After sample homogenization incubate the sample at 65° C for 10 minutes vortexing occasionally or use a shaking thermomixer.
4. Add 130 µL of **Precipitation Solution** and mix by inverting the tube 2-3 times. Incubate 5 minutes on ice. To clear the plant lysate, centrifuge the samples for 10 minutes at maximum speed (3,000-4,000 × g for microtiter deep well 96 plate or 20,000 × g for microtubes).
5. Transfer 400 µL of supernatant to a clean microtube or microtiter deep well 96 plate, making sure not to disturb the pellet or transfer any debris. Immediately proceed with the genomic DNA isolation **Protocols A-C**.

Instructions for lysis of different plant samples.
To purify genomic DNA from woody, lignified and or polyphenol-rich samples such as branches, twigs, needles, wax-coated leaves (such as laurel) and wheat flour, supplement the **Lysis Buffer A** with polyvinylpyrrolidone (PVP) at a 2% (w/v) final concentration.
To purify genomic DNA from seeds (such as *Brassica napus*), supplement the **Lysis Buffer A** with dithiothreitol (DTT) at a 40 mM final concentration.

* Proceed according homogenizer manufacturer recommendations.

PROTOCOL SELECTION GUIDE

The MagJET Plant Genomic DNA Kit provides optimized protocols for genomic DNA purification from different amounts of starting material (10-100 mg). The kit is compatible with automated and manual sample processing, allowing low- to high-throughput nucleic acid purification workflows.

The following selection guide summarizes the available protocols depending on starting sample weight, throughput and sample processing method. Automation protocols are optimized for KingFisher Flex and KingFisher Duo instruments.

Note. Transfer the **Plant_gDNA_Flex** protocol file to the KingFisher Flex instrument or the **Plant_gDNA_Duo** protocol file to the KingFisher Duo before first use. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the BindIt Software for KingFisher Instruments version 3.2 User Manual. The protocol files for MagJET Plant Genomic DNA Kit can be found on product web page on www.thermoscientific.com/onebio

Protocol selection guide:

Plant homogenization type	**Sample quantity**	**Throughput per run**	**KingFisher Flex Instrument**	**KingFisher Duo Instrument**	**Manual processing**	**MagJET purification protocol**	**Page**
Plant homogenized with liquid nitrogen (see the detailed instruction on page 7)	10-100 mg (up to 20 mg of dried plant)	Up to 48	●	-	-	**Protocol A**	page 9
		Up to 12	-	●	-	**Protocol B**	page 10
		variable	-	-	●	**Protocol C**	page 11
Plant homogenized with equipment for disrupting plant tissue* (see the detailed instruction on page 8)	10-50 mg (up to 10 mg of dried plant)	Up to 96	●	-	-	**Protocol A**	page 9
		Up to 12	-	●	-	**Protocol B**	page 10
		variable	-	-	●	**Protocol C**	page 11

***** Sample quantity may depend on the homogenizer type and throughput. Please proceed according homogenizer manufacturer recommendations.

GENOMIC DNA PURIFICATION PROTOCOLS AND PIPETTING INSTRUCTIONS

Protocol A. Instructions for genomic DNA purification using KingFisher Flex 96 and Microtiter deep well 96 plates

Note:

- When using the MagJET Plant Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the **Plant_gDNA_Flex** protocol file to the KingFisher Flex instrument as described on page 8.

1. Homogenize the samples and prepare plant lysate according to the instructions given in "Starting material handling and sample homogenization" on page 6-7.
2. Obtain three empty Thermo Scientific Microtiter deep well 96 plates and two empty Thermo Scientific KingFisher Flex 96 KF plates.
3. Fill the plates as follows:

Plate number	Plate type	Plate name	Content	Volume per well
2	Microtiter deep well 96 plate	Wash 1	Wash Buffer 1 *(supplemented with ethanol)*	800 µL
3		Wash 2_1	Wash Buffer 2 *(supplemented with ethanol)*	800 µL
4		Wash 2_2	Wash Buffer 2 *(supplemented with ethanol)*	800 µL
5	KingFisher Flex 96 KF plate	Elution	Elution Buffer	150 µL
6		Tip plate	-	-

4. Prepare the **Sample** plate.

Add the following reagents to the **Sample** plate: plant lysate, **MagJET Magnetic Beads** and 96% Ethanol.

Plate number	Plate type	Plate name	Content	Volume per well
1	Microtiter deep well 96 plate	Sample	Plant lysate	400 µL
			MagJET Magnetic Beads*	25 µL
			Ethanol 96%	400 µL

* Resuspend **MagJET Magnetic Beads** well by vortexing before use.

5. Place a Thermo Scientific KingFisher Flex 96 tip comb for deep well magnets on a **Tip Plate** (an empty KingFisher Flex 96 KF plate).
6. Swich on the KingFisher Flex 96 and start the **Plant_gDNA_Flex** protocol. Insert the **Tip Plate** and the filled plates into the instrument as indicated on the KingFisher Flex display. After all plates have been loaded into the instrument, the protocol will begin.
7. After the run is finished, remove the plates according to the instructions on the KingFisher Flex display and turn off the instrument. Transfer the eluate (which contains the purified genomic DNA) to a clean new tube or plate.
8. Store the purified DNA at -20°C. The purified DNA is ready for use in downstream applications.

Protocol B. Instructions for genomic DNA purification using KingFisher Duo with 12-pin magnet head and Microtiter deep well 96 plate

Note:

- When using the MagJET Plant Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.
- Transfer the **Plant_gDNA_Duo** protocol file to the KingFisher Duo instrument as described on page 8.
- Ensure you are using the KingFisher Duo 12-pin magnet head and heating block.

1. Homogenize the samples and prepare plant lysate according to the instructions given in "Starting material handling and sample homogenization" on page 6-7.
2. Add the following reagents to the Microtiter deep well 96 plate **row A**: plant lysate, **MagJET Magnetic Beads** and 96% Ethanol (see below).
3. Add the following reagents to the rows. Note that **row B** is reserved for the tips and should be left empty. Note that rows C, D and H are left empty.

Plate name and type	Row	Row name	Content	Volume per well
Plant DNA plate Microtiter deep well 96 plate	A	Sample	Plant lysate	400 µL
			MagJET Magnetic Beads*	25 µL
			Ethanol 96%	400 µL
	B	Tip	12-tip comb	N/A
	C	Empty	Empty	Empty
	D	Empty	Empty	Empty
	E	Wash 1_1	Wash Buffer 1 *(supplemented with ethanol)*	800 µL
	F	Wash 1_2	Wash Buffer 2 *(supplemented with ethanol)*	800 µL
	G	Wash 2	Wash Buffer 2 *(supplemented with ethanol)*	800 µL
	H	Empty	Empty	Empty

* Resuspend **MagJET Magnetic Beads** well by vortexing before use.

4. Prepare the KingFisher Duo **Elution Strip** as follows.

Elution strip	Content	Reagent volume per well
KingFisher Duo elution strip	Elution Buffer	100

5. Place a Thermo Scientific KingFisher Duo 12-tip comb into **row B** on the Plant DNA plate.
6. Switch on the KingFisher Duo instrument and start the **Plant_gDNA_Duo** protocol. Insert the **Plant DNA plate** and **Elution Strip** into the instrument as indicated on the KingFisher Duo display and press **OK**. Make sure that the **Elution Strip** is placed in the correct direction into the elution block; ensure that the perforated end is facing towards the user.
7. When the protocol is completed, remove the plate and **Elution Strip** according to the instructions on the KingFisher Duo display and turn off the instrument. Transfer the eluate (which contains the purified genomic DNA) to a clean tube. Store the purified DNA at -20°C. The purified DNA is ready for use in downstream applications.

Protocol C. Instructions for manual genomic DNA purification

Following protocol is based on transfer of liquids by pipetting through different purification steps rather than magnetic bead transfer as in KingFisher automatic protocols. This allows the kit to be used in various throughput applications using magnetic rack and manual or automated pipetting equipment. Protocols for the other automated pipetting platforms should be optimized for each platform and sample used. To enable protocol optimization all buffers are available to purchase separately.

Note:

- When using the MagJET Plant Genomic DNA Kit for the first time, prepare working solutions of Wash Buffer 1 and Wash Buffer 2 as described on page 5.

1. Homogenize the samples and prepare plant lysate according to the instructions given in "Starting material handling and sample homogenization" on page 7-8.
2. Add 25 µL **MagJET Magnetic Beads resuspended well by vortexing** to 400 µL of the cleared plant lysate.
3. **Binding to magnetic beads:** Add 400 µL 96% ethanol and mix by vortexing for 20 seconds to obtain a uniform suspension.Spin down the tubes to collect all drops from the walls of the tube. Incubate for 1 minute at room temperature and then place microcentrifuge tube in the magnetic particle processing rack and let the **MagJET Magnetic Beads** collect at the magnet for 1-2 minutes or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack, remove and discard the supernatant carefully. Make sure that all supernatant is removed.
4. **Wash 1:** Add 800 µL of **Wash buffer 1** (supplemented with ethanol). Mix by vortexing for 20 seconds to obtain a uniform suspension. Spin down the tube to collect all the drops from the walls of the tube. Incubate for 1 minute at room temperature and then place microcentrifuge tube in the magnetic particle processing rack for 1 minute or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack, remove and discard the supernatant carefully. Make sure that all wash solution is removed.
5. **Wash 2:** Add 800 µL of **Wash buffer 2** (supplemented with ethanol). Mix by vortexing for 20 seconds to obtain a uniform suspension. Spin down the tube to collect all the drops from the walls of the tube. Incubate for 1 minute at room temperature and then place microcentrifuge tube in the magnetic particle processing rack for 1 minute or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack, remove and discard the supernatant carefully. Make sure that all wash solution is removed.
6. **Wash 3:** Repeat step 5.
7. **Dry:** Eliminate the residual ethanol by opening the lid of the tube for 5 minutes at 37°C to evaporate the ethanol completely.
8. **Elution:** Add 150 µL* of **Elution Buffer**. Mix thoroughly by vortexing. Spin down the tube to collect all the drops from the walls. Incubate tubes in thermomixer at 70°C, 600-700 rpm for 5 minutes. Spin down the tube to collect all the drops from the walls of the tube. Place the tube in the magnetic rack for 5 minutes or until the beads have formed a tight pellet. Without removing the microcentrifuge tube from the magnetic rack, carefully transfer eluate (containing DNA) to a clean microcentrifuge tube. Store the purified DNA at -20°C. The purified DNA is ready for use in downstream applications

Note: If more concentrated DNA is required, the volume of **Elution**, can be reduced to 50 µL. If less concentrated DNA is required the volume of **Elution Buffer** can be increased to 200 µL.

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified DNA	**Excess sample used during lysate preparation:** reduce the amount of starting material. Do not use more plant tissue than indicated in lysis protocols. **Insufficient homogenization of plant**. To disrupt the cell wall it is important to homogenize the sample thoroughly until it is ground to fine powder. Efficient homogenization and lysis of the plant increases the DNA yield. **Ethanol was not added to Wash Buffer(s):** make sure that ethanol was added to Wash Buffer 1 and Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on p.5. **Sample storage**. Prolonged storage of the sample material may reduce the DNA yield. **Elution volume**. There should be an adequate volume of the Elution Buffer to cover the MagJET Magnetic Beads completely during the elution step.
Low purity	**Sample storage**. Prolonged storage of the sample material may reduce the quality of the DNA. **Magnetic Beads** Carryover of MagJET Magnetic Beads to the Elution Buffer may affect the A_{260}/A_{280} ratio.
Purified DNA is degraded	**Sample was frozen and thawed repeatedly:** avoid repeated freeze / thaw cycles of the samples. Use a new sample for DNA isolation. Perform extractions from fresh material when possible. **Sample material was not properly mixed with Lysis Buffer A:** all ground material must be thoroughly mixed with the Lysis Buffer A. DNA degradation can occur in particles that are left to dry on the walls of the tube.
RNA contamination	**RNase A treatment was not carried out:** carry out RNase A treatment step described in the purification procedure.
Low $A_{260/280}$ ratio from UV measurement	**Some magnetic particles are left in the elution**: centrifuge eluate at full speed for 1 minute and transfer supernatant to a new tube.
Inhibition of Downstream enzymatic reactions	**Purified DNA contains residual salt:** use the correct order for the Wash Buffers. Always wash the MagJET Magnetic Beads with Wash Buffer 1 first and then proceed with Wash Buffer 2.

Wash buffer 1 for MagJET Plant Genomic DNA Kit

Xn Harmful
Hazard-determining components of labelling: guanidinium chloride

Risk phrases
22 Harmful if swallowed.
36/38 Irritating to eyes and skin.

Safety phrases
23 Do not breathe gas/fumes/vapour/spray.
26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
36/37 Wear suitable protective clothing and gloves.
60 This material and its container must be disposed of as hazardous waste.

PRODUCT USE LIMITATION
This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.