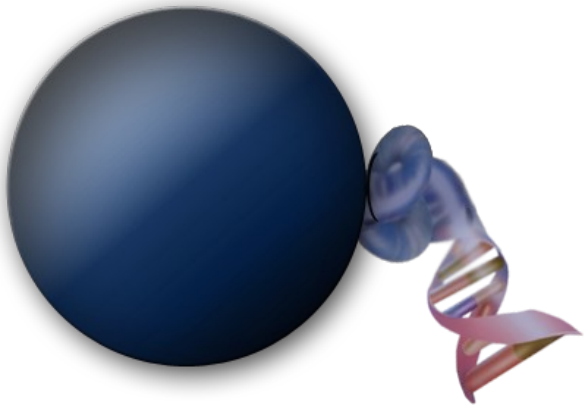
magtivio

MagSi-DNA Vegetal II

Art.No.

MDKT00160096

MDKT00160960



Product Manual

Version 2.0 | 02/12/2019

Table of Contents

1. General Information

1.1 Intended Use

MagSi-DNA Vegetal II is intended for Research Use Only (RUO). The kit is suited for qualified personnel only.

The kit was optimized to extract DNA from plant samples with the highest purity. The extraction chemistry was specifically developed for use with plant samples rich in fats and oils, especially seeds. While many plant DNA extractions require a dilution to further eliminate PCR inhibition, DNA isolated with MagSi-DNA Vegetal II is directly usable in downstream analysis.

The kit is intended for manual and automated isolation of genomic DNA from plant samples. Processing time for DNA extraction from 96 plant lysates is about 30 minutes. The kit requires no phenol/chloroform extraction or ethanol precipitation and eliminates the need for repeated centrifugation, vacuum filtration or column separation. It allows safe handling of samples, and is designed to avoid sample-to-sample cross-contaminations.

MagSi-DNA Vegetal II is suitable for automation on most liquid handling robots. The total processing time depends on the throughput and configuration of the instrument. The beads are easy to handle, have a high binding capacity and enable incubation without intensive mixing.

1.2 Kit specifications

The kit provides reagents for extraction of DNA from up to 10 plant seeds depending on the size, 20-50 mg fresh plant leaf or up to 10 mg lyophilized plant leaf. Purified DNA samples can be stored at 2-8°C. For long-term use, storage at -20°C is recommended. To maintain the high-molecular weight nature of the isolated DNA, it is recommended to avoid freeze-thaw cycles. Stability of lysed plant samples is dependent on the plant species. Lysed samples are typically stable for at least one day at RT, but it is recommended to proceed with DNA extraction immediately.

1.3 Principle of operation

Plant tissue is disrupted by mechanical homogenization and plant cell contents are released with Lysis Buffer VG containing chaotropic salts and detergents. Lysed samples should be cleared by centrifugation in order to remove cellular debris. By adding MagSi-VG III magnetic beads and adjusting binding conditions by addition of Binding Buffer U1, DNA binds to the magnetic beads while leaving impurities in solution. After magnetic separation and removal of the supernatant, the beads are washed three times to remove any residual contaminants and potential PCR inhibitors. A drying step makes sure all traces of ethanol are removed. Finally, purified DNA is eluted off the beads with Elution Buffer and can directly be used for downstream applications.

2. Materials

2.1 Kit Contents

	96 preps MDKT00160096	10 x 96 preps MDKT00160960
Lysis Buffer VG	50 mL	500 mL
Binding Buffer U1	40 mL	400 mL
MagSi-VG III	3 mL	30 mL
Wash Buffer I	60 mL	600 mL
Wash Buffer II	60 mL	600 mL
Elution Buffer	20 mL	200 mL
Manual	1	1

**For DNA extraction without a drying step, Wash Buffer III can be ordered separately (Art.No. MD70041). Please contact magtivio customer support at support@magtivio.com for further information.*

2.2 Reagents, consumables and equipment to be supplied by the user

Reagents

- 80% ethanol
- Proteinase K (10 mg/mL) (optional), 10 µL per preparation
- RNase (10 mg/mL) (optional), 10 µL per preparation

Consumables and equipment for manual or automated processing

Product	Manual use	Automated use
Sample containers	1.5 or 2 mL microtubes	Recommended: Riplate®SW 96, PP, 2ml, (Ritter, 43001–0020) Nunc™ 96-well Polypropylene DeepWell™ Storage Plate 2.0mL, (Thermo Scientific, Cat.No. 278752)
Magnetic separation	MM-Separator M12 + 12 P Art.No. MDMG0001	MM-Separator 96 DeepWell Art.No. MDMG0013
Final container	1.5 or 2 mL microtubes	96-well microplate
Tissue homogenization	Commercial homogenizers, e.g. Geno/Grinder or TissueLyser	
Mixing	Tube Vortexer	Microplate shaker (min. 1000 RPM)
Heating	Incubator or water bath for plant cell lysis	

Consumables for processing on the KingFisher Flex instrument

Product	Art. No.	Contents
2 ml Deepwell Plate with square wells for KingFisher™	MDPL00200060	60 pieces
200 µL square-well Elution Plate for KingFisher™	MDPL00190060	60 pieces
96 well Tip-Comb for KingFisher™	MDPL00210060	60 pieces

3. Kit usage

3.1 Storage Conditions

All components of the MagSi-DNA Vegetal II kit can be stored at room temperature (18-25°C). The kit is stable for up to 1 year, but no longer than the expiry date on the label.

3.2 Preparation of reagents

- If there is any precipitate present in the buffers, warm the buffer to 25-37°C to dissolve the precipitate before use.
- Using absolute ethanol, dilute with appropriate water to 80%. Prepare at least 60 mL 80% ethanol per 96 samples.
- Immediately before use, resuspend MagSi-VG III by vortexing for 20 seconds.

3.3 Safety instructions

Take appropriate safety measures, such as wearing a suitable lab coat, disposable gloves, and protective goggles. Follow local legal requirements for working with biological materials. More information is found in the safety data sheets (SDS), available at www.magtivio.com under each magtivio kit and kit component.

Infectious potential of liquid waste left over after using MagSi-DNA Vegetal II was not tested. Even though contamination of waste with residual infectious material is unlikely, it cannot be excluded completely. Therefore, liquid waste should be handled as being potentially infectious, and discarded according to local safety regulations.

3.4 Considerations

1. It is recommended to use young plant tissue samples and keep plants in the dark to reduce polysaccharide content. In many cases lyophilized, dried material can be processed more easily and gives higher yield. Depending on plant species and sample type (seeds / leafs), the volume of the Lysis Buffer VG can be optimized. The lysis process is most efficient when using well homogenized sample material. We recommend the use of commercial homogenizers.

2. In some cases, lysis efficiency can be improved by addition of 10 µL Proteinase K (10 mg/mL).

3. If samples contain large amounts of RNA, it is recommend to add 10 µL RNase A (10 mg/mL) to the lysis mixture before incubation.

4. Elution can be performed at room temperature. Yields may be increased if elution is performed at 60°C. In most consumables elution can be carried out in ≥50 µL. Do not use less than the minimum working volume of the container used as it is essential to completely submerge the beads in elution buffer during the elution step and in order to allow magnetic separation. For some separators and sample containers, higher or lower elution volumes may be needed to contact the whole magnetic bead pellet. To avoid cross-contamination and DNA degradation, change pipette tips after each use and use nuclease-free filter-tips.

5. Lysis efficiency and DNA yield are highly dependent on the sample type. Different volumes of Lysis Buffer VG can be used to increase DNA yields. Conditions for binding have to be adjusted by taking a volume of Binding Buffer U1 that is at least equal to the volume of lysate transferred after centrifugation.

3.5 Magnetic Separation systems

MagSi-DNA Vegetal II has been designed for use on the MM-Separator 96 DeepWell and MM-Separator M12 + 12 P. The MM-Separator M12 + 12 P (Art.No. MDMG0001) allows simultaneous processing of up to 12 samples in 2 mL microcentrifuge tubes. For processing in 96 deepwell plates, use the MM-Separator 96 DeepWell (Art.No. MDMG0013).

For use with other magnetic separators, please contact the customer support at support@magtivio.com.

MagSi-DNA Vegetal II is compatible with KingFisher™ Flex Magnetic Particle Processor by Thermo Scientific™. Protocols and consumables are available on request.

3.6 Shaker settings

The speed settings for the microplate shaker described in the protocols that follow were defined with a specific instrument and microplate. When first using a plate shaker for incubation steps, the speed settings have to be set carefully for each specific plate to prevent cross contamination and spillage. Setting the speed of the shaker can be done by loading a microplate with a volume of dyed water equal to the working volume during each step, and step-wise increasing the shaker speed until droplets are observed on the surface of the plate. Set the shaker speed lower again.

3.7 Product use limitations

MagSi-DNA Vegetal II is intended for research use only. Do not use for other purposes than intended. The kit components can be used only once. Do not combine components of different kits unless the lot numbers are identical. Avoid leaving bottles open to prevent contamination or evaporation of the kit reagents. Process only as many plant samples in parallel as the magnetic separator allows.

4. Protocols

4.1 Manual DNA extraction from plant samples (with drying step)

Before starting:

- *Using molecular biology grade ethanol, dilute with appropriate water to 80%*
- *Immediately before use, resuspend MagSi-VG III by vortexing for 20 seconds*

1. Homogenize up to **50 mg** fresh plant sample (or <10 mg lyophilized plant sample) by mechanical disruption.
2. Add **500 µL Lysis Buffer VG** (preheated) and incubate the samples at **65°C** for **30 min**.

   Optional: if samples contain large amounts of RNA or if samples need to be RNA-free, we recommend to add 10 µL RNase A (~10 mg/mL) to the lysis mixture.
3. Centrifuge for **15 min** (**>6.000 x *g***) to pellet contaminants and cell debris. Transfer **400 µL cleared lysate** to a deepwell microplate or microtube.
4. Add **400 µL Binding Buffer U1** and **30 µL MagSi-VG III**. Incubate on a microplate shaker for 5 min at 1000 RPM.
5. Place the samples on the magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
6. Remove the sample plate from the magnetic separator and add **600 µL Wash Buffer I** to the tubes. Incubate on a microplate shaker for 1 min at 1000 RPM. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
7. Repeat step 6 one more time with **600 µL Wash Buffer II** and one time with **600 µL 80% ethanol**.
8. Dry the beads on air for **10 min** to evaporate the ethanol completely.
9. Remove the sample plate from the magnetic separator and add **50-200 µL Elution Buffer**. Incubate on a microplate shaker for 5 min at 1000 RPM.
10. Place the samples on the magnetic separator and wait for 1 minute to collect the beads. Transfer the eluates to new tubes. The DNA in the eluate is now ready to use.

    - *If the transferred eluates contain magnetic particles, place the tubes on the magnetic separator again, separate for 1 minute and transfer the eluates to new tubes.*
    - *The DNA can be eluted with different volumes of Elution Buffer (depending on the required volume for subsequent analysis).*

4.2 Manual DNA extraction from plant samples (with WB III)

Before starting:

- *Using molecular biology grade ethanol, dilute with appropriate water to 80%*
- *Immediately before use, resuspend MagSi-VG III by vortexing for 20 seconds*

1. Homogenize up to **50 mg** fresh plant sample (or <10 mg lyophilized plant sample) by mechanical disruption.
2. Add **500 µL Lysis Buffer VG** (preheated) and incubate the samples at **65°C** for **30 min**.

   Optional: if samples contain large amounts of RNA or if samples need to be RNA-free, we recommend to add 10 µL RNase A (~10 mg/mL) to the lysis mixture.
3. Centrifuge for **15 min** (**>6.000 x *g***) to pellet contaminants and cell debris. Transfer **400 µL cleared lysate** to a deepwell microplate or microtube.
4. Add **400 µL Binding Buffer U1** and **30 µL MagSi-VG III**. Incubate on a microplate shaker for 5 min at 1000 RPM.
5. Place the samples on the magnetic separator and wait for 1 min to collect the beads. Remove supernatants.
6. Remove the sample plate from the magnetic separator and add **600 µL Wash Buffer I** to the tubes. Incubate on a microplate shaker for 1 min at 1000 RPM. Place the tubes in a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
7. Repeat step 6 one more time with **600 µL Wash Buffer II** and one time with **600 µL 80% ethanol**.
8. With the samples on the magnet, slowly add **800 µL Wash Buffer III**. Wait for 30 seconds and carefully remove the supernatant again. Do not resuspend beads and do not exceed 60 seconds as this may cause early DNA elution. When using the kit manually, it is recommended to not treat samples with Wash Buffer III simultaneously.
9. Remove the sample plate from the magnetic separator and add **50-200 µL Elution Buffer**. Incubate on a microplate shaker for 5 min at 1000 RPM.
10. Place the samples on the magnetic separator and wait for 1 minute to collect the beads. Transfer the eluates to new tubes. The DNA in the eluate is now ready to use.

    - *If the transferred eluates contain magnetic particles, place the tubes on the magnetic separator again, separate for 1 minute and transfer the eluates to new tubes.*
    - *The DNA can be eluted with different volumes of Elution Buffer (depending on the required volume for subsequent analysis).*

4.3 DNA extraction from plant samples on the KingFisher Flex™

4.3.1 KingFisher BindIt sofware protocol

Please contact magtivio for the most recent BindIt software method files. We provide the corresponding files for direct upload on the KingFisher magnetic particle processors. A PDF description of the method file is included. Refer to the BindIt software manual regarding the upload procedure of the supplied software files to the instrument.

4.3.2 Homogenization and lysis

1. Homogenize up to **50 mg** fresh plant sample (or <10 mg lyophilized plant sample) by mechanical disruption.
2. Add **500 µL Lysis Buffer VG** (preheated) and incubate the samples at **65°C** for **30 min**.

   Optional: if samples contain large amounts of RNA or if samples need to be RNA-free, we recommend to add 10 µL RNase A (~10 mg/mL) to the lysis mixture.

3. Centrifuge for **15 min** (**>6.000 x *g***) to pellet contaminants and cell debris. Transfer **400 µL cleared lysate** to a 2 ml Deepwell Plate with square wells for KingFisher™.

4.3.3 Preparation of processing plates

Initial plate filling for instrument set-up:

Plate	Type*)	Reagent	Volume
Sample Plate	2 ml Deepwell Plate with square wells for KingFisher™	Plant lysate Binding Buffer U1 MagSi-VG III	400 µL 400 µL 30 µL
Wash Buffer I	2 ml Deepwell Plate with square wells for KingFisher™	Wash Buffer I	600 µL
Wash Buffer II	2 ml Deepwell Plate with square wells for KingFisher™	Wash Buffer II	600 µL
80% ethanol	2 ml Deepwell Plate with square wells for KingFisher™	80% ethanol	600 µL
Elution Buffer	200 µL square-well Elution Plate for KingFisher™	Elution Buffer	150 µL
Tip plate	2 ml Deepwell Plate with square wells for KingFisher™	Empty, for loading Tip-Comb only	N/A

*) We strongly recommend to use only the plates which are intended to use on the KingFisher magnetic particle processor. Using unsuitable plates may result in extraction failure or instrument damage.

4.3.4 Detailed instructions

Follow exactly the instructions as given below. Label all plates thoroughly and unambiguously to avoid any misloading during the instrument loading procedure.

1. Prepare one plate for the binding step with MagSi-VG III and Binding Buffer U1. To each well of the Sample Plate already containing 400 µL plant lysate, dispense 30 µL **MagSi-VG III** magnetic beads and 400 µL **Binding Buffer U1**.

2. Prepare one plate for the 1st wash step with **Wash Buffer I.** Add 600 µL **Wash Buffer I** to each well of the corresponding deep-well plate.

3. Prepare one plate for the 2nd wash step with **Wash Buffer II**. Add 600 µL **Wash Buffer II** to each well of the corresponding deep-well plate.

4. Prepare one plate for the 3rd wash step with **80% ethanol**. Add 600 µL **80% ethanol** to each well of the corresponding deep-well plate.

5. Prepare one plate for **Elution Buffer**. Add 150 µL **Elution Buffer** to each well of the corresponding deep-well plate.

6. Switch on the KingFisher Flex magnetic particle processor and select the **"MagSi-DNA-Vegetal-II"** protocol from the user defined protocols

7. Start the protocol.

8. Load the plates to the instrument, following the instructions on the instrument display. The order of plates starts with the tip plate and ends with the sample plate.

   Make sure that all plates are inserted in the same orientation (especially when using partially filled plates). Place the A1 well of each plate to the A1 mark on the instruments turntable.

9. At the end of the method remove all plates from the instrument. Follow the instructions on the instrument display.

5. Troubleshooting

Problem	Possible causes	Comments and suggestions
Low DNA yield	Sample contains too low or too high amounts of plant material	- Try using larger or smaller amounts of plant material
	Incomplete lysis	- Increase incubation time for lysis - Make sure Lysis Buffer VG does not contain precipitates - Add Proteinase K (10 µL, 10 mg/mL) to the sample before incubation at 65°C
	Inefficient binding to the magnetic particles	- Use correct amounts of all reagents - Make sure the microplate shaker speed is set appropriately (see section 3.6) - Increase binding time - If samples contain large amounts of RNA, add RNase A to the lysis mixture before incubation at 65°C
	Incomplete elution	- Increase drying time for evaporation of ethanol - Increase elution time from 5 to 10 minutes - Preheat Elution Buffer to 60°C before use - Perform elution at 60°C to increase elution efficiency - Try eluting twice with 100 µL Elution Buffer
	Incomplete collection of magnetic particles	- Prolong the time-to-magnet after binding step and washing steps
Degraded or sheared DNA	Incorrect storage of the sample material	- Sample should be harvested, stored and homogenized properly - Avoid repeated thawing and freezing
Problems in downstream applications / contamination in DNA sample	Ethanol in the eluted DNA	- Increase the evaporation time for Wash Buffer II - Replace drying step with Wash Buffer III (Section 4.2)
	Salt in the eluate (high adsorption at 230 nm)	- Make sure that wash supernatants are efficiently removed - Wash Buffers should be stored and used at RT - Repeat washing step with Wash Buffer II - Replace drying step with Wash Buffer III (Section 4.2)
	Magnetic beads remaining in the eluate	- Place the DNA eluates in the magnetic separator again, and transfer the supernatant to a new container.

magtivio B.V.

Office:
Vlotstraat 2-4
6417 CB Heerlen (The Netherlands)
Tel: +31-(0)46-820 0206
Fax: +31-(0)46-410 6825
E-mail: info@magtivio.com

Lab & Production:
De Asselen Kuil 12a
6161 RD Geleen (The Netherlands)

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