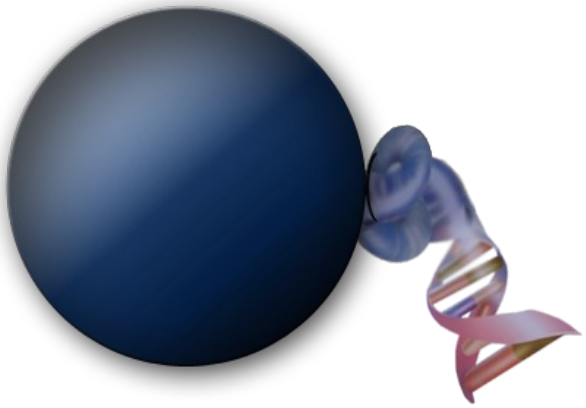
magtivio

MagSi-DNA Vegetal

Art.No.

MDKT0005



Product Manual

Version 1.0 | 08/08/2018

Table of Contents

1. General Information

1.1 Intended Use

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

MagSi-DNA Vegetal is designed for manual and automated isolation of genomic DNA from plant tissues. 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 samples, and is designed to avoid sample-to-sample cross-contaminations.

The kit is designed for use with MM-Separator M12+12 P or other magnetic separation systems (see section 2.2). Manual time for DNA extraction of 96 samples is about 120 minutes. The purified DNA is ready-to-use for subsequent detection methods, especially PCR technologies. The procedure is optimized for 20-50 mg plant material. Reagent volumes can be scaled up or down to be used for different sample amounts.

MagSi-DNA Vegetal 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 Principle of operation

Plant tissue is extracted with Lysis Buffer VG containing chaotropic salts and detergents. Lysed samples should be cleared by centrifugation in order to remove cellular debris. By transferring the supernatant, adding magnetic beads and adjusting binding conditions by addition of Binding Buffer VG, DNA binds to the magnetic beads. After magnetic separation and removal of the supernatant, the beads are washed three times to remove 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

Kit size Article Number	96 preps MDKT00050096	10 x 96 preps MDKT00050960
Lysis Buffer VG	50 mL	500 mL
Binding Buffer VG	50 mL	500 mL
MagSi-VG I*	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

**MagSi-VG I beads can be replaced with MagSi-VG II beads for specific automation requirements. Contact magtivio customer support at support@magtivio.com for further information.*

**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

Product	Manual use	Automated use
Sample containers	2 mL microcentrifuge tubes	96 deep-well microplate (2.1 mL)
Magnetic separation	MM-Separator M12 + 12 P (MDMG0001)	MM-Separator 96 DeepWell (MDMG0013)
Final container	2 mL microtubes	96-well microplate
Homogenization	Commercial homogenizers, e.g. Geno/Grinder or TissueLyser	
Mixing	Vortexer	Plate shaker
Heating	Incubator or water bath for preheating Lysis Buffer VG and the Elution Buffer	

3. Kit usage

3.1 Storage Conditions

All components of MagSi-DNA Vegetal 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.
- Preheat Lysis Buffer VG and Elution Buffer to 65°C.
- Using molecular biology grade ethanol, dilute with appropriate water to 80%.
- Immediately before use, resuspended MagSi-VG I 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 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 and incubate for 10-20 minutes at 37°C.

4. Elution can be performed at room temperature. Yields may be increased if elution is performed at 60°C. Elution can be carried out in ≥50 µL. It is essential to completely submerge the beads in elution buffer during the elution step. For some separators, 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 VG equal to the volume of lysate transferred after centrifugation.

3.5 Magnetic Separation systems

MagSi-DNA Vegetal has been designed for use on the MM-Separator M12 + 12 P and MM-Separator 96 DeepWell. 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.

3.6 Shaker settings

When 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 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. Protocol for the isolation of genomic DNA from plant samples

Before starting:

- *Preheat Vegetal Lysis Buffer VG and Elution Buffer to 65°C.*
- *Using molecular biology grade ethanol, dilute with appropriate water to 80%.*
- *Immediately before use, resuspend MagSi-VG I by vortexing for 20 seconds.*

1. Homogenize **20-50 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 RNase A (12 mg/mL) to the lysis mixture.
3. Centrifuge for **15 min** (**>6.000 x *g***) to pellet contaminants and cell debris. Transfer **400 µL of the cleared lysate**.
4. Vortex and add **30 µL MagSi-VG I** and **400 µL Binding Buffer VG** and to each sample and mix the samples by pipetting up and down 5 times and incubate on a shaker for **5 min**. Alternatively, when processing without a shaker, mix by pipetting up and down 20 times and incubate at room temperature for 5 min. Place the samples on a magnetic separator and wait for **1 min** to collect the beads. Remove and discard supernatants by pipetting.
5. Add **600 µL Wash Buffer I** to the samples and mix the samples on a shaker until beads are completely resuspended (**3 min**). Alternatively, mix by pipetting up and down 15 times to resuspend beads. Place the samples on a magnetic separator and wait for **1 min** to collect the beads. Remove and discard supernatants by pipetting.
6. Add **600 µL Wash Buffer II** to the samples and mix the samples on a shaker until beads are completely resuspended (**3 min**). Alternatively, mix by pipetting up and down 15 times to resuspend beads. Place the samples on a magnetic separator and wait for **1 min** to collect the beads. Remove and discard supernatants by pipetting.
7. Add **600 µL ethanol 80%** to the samples and mix the samples on a shaker until beads are completely resuspended (**3 min**). Alternatively, mix by pipetting up and down 15 times to resuspend beads. Place the samples on a magnetic separator and wait for **1 min** to collect the beads. Remove and discard supernatants by pipetting.
8. Dry the beads on air for **10 min** to evaporate the ethanol completely. *Alternatively, drying can be replaced by soaking the wells with 400 µL (non-alcohol) Wash Buffer III (available separately: Art.No. MD71041 / MD72041):*

   Add **400 µL Wash Buffer III** to the samples, wait 30 seconds and remove the buffer again.

   Important note: It is critical that the beads remain on the magnet, do not mix or try to resuspend the beads! Do not exceed 45 seconds of exposure to Wash Buffer III as this may lead to loss of DNA.
9. Add **50-200 µL Elution Buffer** and mix the samples by pipetting up and down 5 times and incubate on a shaker for **5 min**. Alternatively, when processing without a shaker, mix by pipetting up and down 20 times and incubate at room temperature for 5 min. Place the samples on the magnetic separator and wait for **1 min** 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).*
- *Heating of samples at 60°C during elution can increase DNA yields up to 20%. The DNA can be eluted with a lower volume of Vegetal Elution Buffer (depending on the expected yield of genomic DNA).*

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 to the lysis mixture before incubation at 60°C
	Inefficient binding to the magnetic particles	- Use correct amounts of all reagents - Increase mixing steps after adding Binding Buffer VG - Increase binding time - Mix on a shaker during binding incubation - If samples contain large amounts of RNA, add Rnase A to the lysis mixture before incubation at 60°C
	Incomplete elution	- Increase drying time for evaporation of ethanol - 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 VG
	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 and stored properly - Avoid repeated thawing and freezing of blood sample
Problems in downstream applications / contamination in DNA sample	Ethanol in the eluted DNA	- Increase the evaporation time for Wash Buffer II - Eliminate the drying step by use of Wash Buffer III
	Salt in the eluate (high adsorption at 230 nm)	- Make sure that supernatants are completely removed. - Wash Buffers should be stored and used at RT - Repeat washing step with Wash Buffer II
	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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