

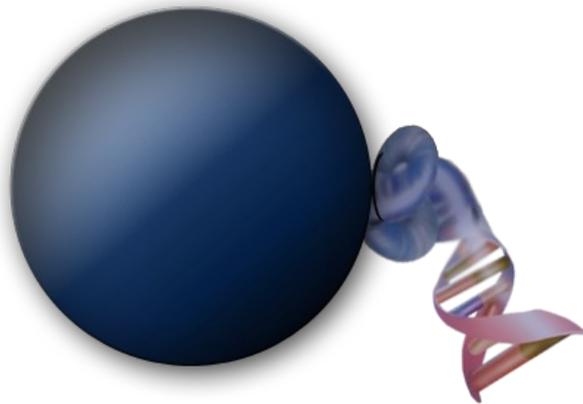
magtivio

MagSi-DNA Vegetal III

Art.No.

MDKT00190096

MDKT00190960



Product Manual

Version 3.0 | 25/02/2020

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1. General Information

1.1 Intended Use

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

The kit is optimized to extract DNA from plant samples with the highest purity and works well with plant leaf samples rich in secondary metabolites. While many plant DNA extractions require a dilution to further eliminate PCR inhibition, DNA isolated with MagSi-DNA Vegetal III 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 III 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 20-50 mg fresh plant leaf or up to 10 mg lyophilized plant leaf, or up to 10 plant seeds depending on the size. 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 CTAB based Lysis Buffer PL containing 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 VG, 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 MDKT00190096 | 10 x 96 preps MDKT00190960 |
|-------------------|---|--------------------------|-------------------------------|
| Lysis Buffer PL | ● | 50 mL | 500 mL |
| Binding Buffer VG | ● | 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, magtivio Art.No. MD), 10 µL per preparation
- RNase (10 mg/mL) (optional), 10 µL per preparation
- Reducing agent, e.g. DTT or TCEP, final 10 mM (optional, add to Lysis Buffer PL immediately before use)

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 III 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 III 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 PL 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 μ L Proteinase K (10 mg/mL).
3. If leaf samples are prone to DNA oxidation, it is recommend to add a reducing agent (e.g. DTT or TCEP, 10 mM final concentration) to the Lysis Buffer PL immediately before use.
4. If samples contain large amounts of RNA, it is recommend to add 10 μ L RNase A (10 mg/mL) to the lysis mixture before incubation.
5. 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 \geq 50 μ L. 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.
6. Lysis efficiency and DNA yield are highly dependent on the sample type. Different volumes of Lysis Buffer PL can be used to increase DNA yields. Conditions for binding have to be adjusted by taking a volume of Binding Buffer VG that is at least equal to the volume of lysate transferred after centrifugation.
7. Lysis Buffer PL is optimized for DNA extraction from plant leaves. For seed samples, Lysis Buffer PL can be exchanged with Lysis Buffer VG, offering a flexible solution for both sample types in a single extraction run.

3.5 Magnetic Separation systems

MagSi-DNA Vegetal III 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 III 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 III 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 PL** ● 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 VG** ● 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 PL** ● 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 VG** ● 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 software 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 PL**  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 VG  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 VG. To each well of the Sample Plate already containing 400 μ L plant lysate, dispense 30 μ L **MagSi-VG III** magnetic beads and 400 μ L **Binding Buffer VG**.
2. Prepare one plate for the 1st wash step with **Wash Buffer I**. Add 600 μ L **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 μ L **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 μ L **80% ethanol** to each well of the corresponding deep-well plate.
5. Prepare one plate for **Elution Buffer**. Add 150 μ L **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-III**" 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 PL 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) |
| | Polyphenol oxidation | - Add reducing agent to Lysis Buffer PL just before use, e.g. DTT or TCEP, 10 mM final concentration |
| | 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, Lab & Production

Daelderweg 9

6361 HK Nuth (The Netherlands)

Tel: +31-(0)45-208 4810

Fax: +31-(0)45-208 4817

E-mail: info@magtivio.com

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