Steinbrenner Laborsysteme GmbH

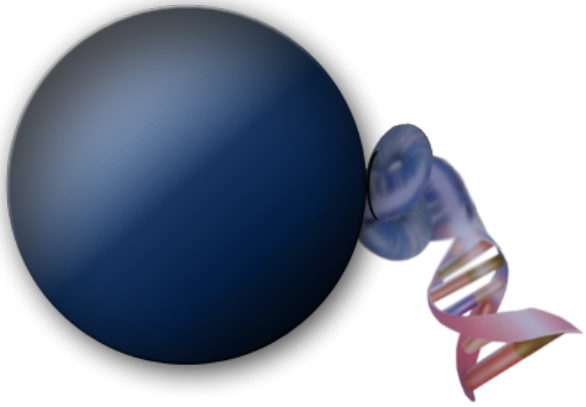
magtivio

MagSi-DNA Plant CLS

Art.No.

MDKT00260096

MDKT00260960



Product Manual

Revision 1.0 | 06-04-2023

Revision history		
Revision	**Release date**	**Remarks**
1.0	06/04/2023	Initial release

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1. General Information

1.1 Intended Use

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

The kit is intended for DNA extraction from plant samples using magnetic beads. The extraction chemistry was developed for optimal results with various sample types such as seeds rich in fats and oils, as well as leaf samples containing secondary metabolites. MagSi-DNA Plant CLS includes 2 lysis buffers offering a flexible solution for different sample types in a single extraction run. Lysis Buffer VG is most suitable for seed samples and Lysis Buffer PL is optimized for DNA extraction from plant leaves. However, due to the variation in sample composition, storage and pretreatment, it is recommended to test any specific sample material with both lysis buffers.

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. While many plant DNA extractions require a dilution to further eliminate PCR inhibition, most DNA isolated with MagSi-DNA Plant CLS is directly usable in downstream analysis.

MagSi-DNA Plant CLS is suitable for automated use on liquid handling workstations and with magnetic particle processors.

1.2 Kit specifications

The kit provides reagents for extraction of DNA from up to 50 mg 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 either Lysis Buffer VG containing SDS or Lysis Buffer PL containing CTAB. Lysed samples should be cleared by centrifugation in order to remove cellular debris. By adding MagSi-PL13 magnetic beads and adjusting binding conditions by addition of Binding Buffer VG, DNA binds to the magnetic beads while leaving most 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

Component	96 preps MDKT00260096	10 x 96 preps MDKT00260960
Lysis Buffer VG	50 mL	500 mL
Lysis Buffer PL	50 mL	500 mL
Binding Buffer VG	40 mL	400 mL
MagSi-PL13	2 mL	20 mL
Wash Buffer I	80 mL	800 mL
Wash Buffer II	2 x 80 mL	2 x 800 mL
Elution Buffer	20 mL	200 mL
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2.2 Reagents, consumables and equipment to be supplied by the user

2.2.1 Reagents (optional)

- Proteinase K (20 mg/mL), 10 µL per preparation (REF: MDRE00130020 / MDRE00130200)
- RNase (10 mg/mL), 10 µL per preparation (REF: MDRE00150040)

2.2.2 Consumables and equipment

Item	Recommended
96 deepwell extraction plate	Riplate®SW 96, PP, 2ml, (Ritter, REF: 43001–0020) Nunc™ 96-well Polypropylene DeepWell™ Storage Plate 2.0mL, (Thermo Scientific, REF: 278752)
96-well elution plate	Nuclease-free microtiter plate
Magnetic separator	MM-Separator 96 DeepWell, REF: MDMG0013
Tissue homogenizer	2010 Geno/Grinder® (SPEX SamplePrep)
Resuspension of MagSi-PL13 beads	Vortex-Genie 2 (Scientific Industries, REF: SI-0236)
Microplate shaker (≥1000 RPM)	Eppendorf ThermoMixer® C (REF: 5382000015)
Heating (Lysis)	Incubator or water bath (≥65°C)
Centrifuge	Depending on plate type used, recommended >6.000 x g

2.2.3 Consumables for processing on the PurePrep 96 System or KingFisher™ Flex instrument

Product	Art. No.	Contents
PurePrep 96 DeepWell Plate	MDPL00200060	60 pieces
PurePrep 96 Elution Plate	MDPL00190060	60 pieces
PurePrep 96 TipComb	MDPL00210060	60 pieces

3. Kit usage

3.1 Storage Conditions

All components of the MagSi-DNA Plant CLS kit can be stored at room temperature (18-25°C). When stored under the conditions mentioned, the kit is stable as indicated by the expiry date on the label.

3.2 Preparation of reagents

All components included are ready-to-use. MagSi-PL13 beads should be well resuspended immediately before use. If there is any precipitate present in the buffers, warm up to 25-37°C to dissolve the precipitate.

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 on request.

Infectious potential of liquid waste leftovers after using MagSi-DNA Plant CLS 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.

2. Depending on plant species and sample type, the volume of lysis buffer can be optimized. The lysis process is most efficient when using well homogenized sample material. We recommend the use of commercial homogenizers.

3. In some cases, lysis efficiency can be improved by addition of 10 µL Proteinase K (20 mg/mL).

4. 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.

5. If leaf samples are prone to DNA oxidation, it is recommended to add a reducing agent (e.g. DTT or TCEP, 10 mM final concentration) to Lysis Buffer VG or Lysis Buffer PL immediately before use.

6. 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 but it is essential to completely submerge the beads in elution buffer during the elution step.

7. To avoid cross-contamination and DNA degradation, it is recommended to change pipette tips after each use and use nuclease-free filter-tips.

3.5 Magnetic Separation systems

MagSi-DNA Plant CLS has been designed for use on the MM-Separator 96 DeepWell (REF: MDMG0013), allowing for processing in 96 deepwell plates.

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

MagSi-DNA Plant CLS is compatible with the PurePrep 96 System and the KingFisher™ Flex Magnetic Particle Processor by Thermo Scientific™. Information of use on these instruments is described in sections 4.2 and 4.3. Software protocol files 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 and use this setting for mixing steps in the extraction procedure.

3.7 Product use limitations

MagSi-DNA Plant CLS 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 use

4.1 Manual DNA extraction from plant samples

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

Note: 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.

*Note: Immediately before use, resuspend **MagSi-PL13** beads by vortexing at maximum speed for 20 seconds*

4. Add **20 µL MagSi-PL13** and **400 µL Binding Buffer VG**. Incubate on a 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 **800 µL Wash Buffer I**. Incubate on a shaker for 1 min at 1000 RPM. Place the sample plate on a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
7. Remove the sample plate from the magnetic separator and add **800 µL Wash Buffer II**. Incubate on a shaker for 1 min at 1000 RPM. Place the sample plate on a magnetic separator and wait for 1 min to collect the beads. Remove the supernatants.
8. Repeat step 7 one more time for a total of 2 washes with **800 µL Wash Buffer II**.
9. Dry the beads on air for 10 min to evaporate the ethanol completely.
10. Remove the sample plate from the magnetic separator and add **50-200 µL Elution Buffer**. Incubate on a shaker for 5 min at 1000 RPM.
11. Place the samples on the magnetic separator and wait for 1 minute to collect the beads. Transfer the eluted DNA samples to a new microplate for subsequent analysis.

Note: If magnetic particles are accidentally transferred, place the plate on the magnetic separator again, separate for 1 minute and transfer the eluates to new tubes.

4.2 Protocol for the PurePrep 96 System

4.2.1 PurePrep 96 software protocol file

Please contact magtivio for the most recent software method files. We provide the corresponding files for direct upload on the PurePrep 96 System. Refer to the PurePrep 96 user manual regarding the upload procedure of the supplied software files to the instrument.

4.2.2 Homogenization and lysis

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

Note: 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 PurePrep 96 Deepwell Plate.

4.2.3 Preparation of processing plates

Plate filling instructions:

Plate name	Plate type	Reagent (Kit component)	Volume	Instrument Position (“Plate”)
Tip plate	PurePrep 96 Deepwell Plate	Empty, for loading Tip-Comb only	N/A	1
Sample Plate	PurePrep 96 Deepwell Plate	Plant lysate Binding Buffer VG MagSi-PL13	400 µL 400 µL 20 µL	2
Wash Plate 1	PurePrep 96 Deepwell Plate	Wash Buffer I	800 µL	3
Wash Plate 2	PurePrep 96 Deepwell Plate	Wash Buffer II	800 µL	4
Wash Plate 3	PurePrep 96 Deepwell Plate	Wash Buffer II	800 µL	5
Elution Plate	PurePrep 96 Elution Plate	Elution Buffer	150 µL	8

Suitable plates can be purchased at magtivio (see section 2.2.3). We strongly recommend using only the plates which are intended to use on the PurePrep 96 System. Using unsuitable plates may result in extraction failure or instrument damage.

4.2.4 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 the “Sample Plate” for the binding step with **MagSi-PL13** and **Binding Buffer VG.** To each well of the Sample Plate already containing **400 µL plant lysate,** dispense **20 µL MagSi-PL13** and **400 µL Binding Buffer VG** ●**.**

2. Prepare “Wash Plate 1” with **Wash Buffer I.** Add **800 µL Wash Buffer I** 🔵 to each well of the corresponding deepwell plate.

3. Prepare “Wash Plate 2” with **Wash Buffer II.** Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deepwell plate.

4. Prepare “Wash Plate 3” with **Wash Buffer II.** Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deepwell plate.

5. Prepare “Elution Plate” with **Elution Buffer.** Add **150 µL Elution Buffer** 🟠 to each well of the corresponding square-well elution plate.

6. Switch on the PurePrep 96 System and select the protocol from the user defined protocols

7. Load all plates to the PurePrep 96 instrument on indicated positions, see section 4.2.3 (right-most column). Use the clockwise / counter clockwise buttons on the instrument to rotate the turntable to the indicated positions.

   Make sure that the plates are loaded in the correct orientation (especially when using partially filled plates). Place the A1 well of each plate to the A1 mark on the instruments turntable. Make sure that the plates are fixed to the positions by the clamps.

8. Press on the Tab “Run Prog.”, select the shortcut icon for the protocol and press Run to start the protocol

9. At the end of the run remove all plates from the instrument

4.3 Protocol for the KingFisher™ Flex Purification System with 96 Deep-Well Head

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 or frozen plant sample (or <10 mg lyophilized plant sample) by mechanical disruption.
2. Add **500 µL Lysis Buffer VG** or **Lysis Buffer PL** and incubate the samples at 65°C for 30 min.

Note: 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 PurePrep 96 Deepwell Plate.

4.3.3 Preparation of processing plates

Plate filling instructions

Plate	Plate type	Reagent (Kit component)	Volume
Sample Plate	PurePrep 96 Deepwell Plate	Plant lysate Binding Buffer VG MagSi-PL 13	400 µL 400 µL 20 µL
Wash Plate 1	PurePrep 96 Deepwell Plate	Wash Buffer I	800 µL
Wash Plate 2	PurePrep 96 Deepwell Plate	Wash Buffer II	800 µL
Wash Plate 3	PurePrep 96 Deepwell Plate	Wash Buffer II	800 µL
Elution Plate	PurePrep 96 Elution Plate	Elution Buffer	150 µL
Tip plate	PurePrep 96 Deepwell Plate	Empty, for loading Tip-Comb only	N/A

Suitable plates can be purchased at magtivio (see section 2.2.3). We strongly recommend using only the plates which are intended to use on the KingFisher Flex™ System. 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 the “Sample Plate” for the binding step with **MagSi-PL13** and **Binding Buffer VG.** To each well of the Sample Plate already containing **400 µL plant lysate,** dispense **20 µL MagSi-PL13** and **400 µL Binding Buffer VG** ⚫**.**
2. Prepare “Wash Plate 1” with **Wash Buffer I.** Add **800 µL Wash Buffer I** 🔵 to each well of the corresponding deepwell plate.
3. Prepare “Wash Plate 2” with **Wash Buffer II.** Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deepwell plate.
4. Prepare “Wash Plate 3” with **Wash Buffer II.** Add **800 µL Wash Buffer II** 🟡 to each well of the corresponding deepwell plate.
5. Prepare “Elution Plate” with **Elution Buffer.** Add **150 µL Elution Buffer** 🟠 to each well of the corresponding square-well elution plate.
6. Switch on the KingFisher Flex magnetic particle processor and select the protocol from the user defined protocols
7. Start the protocol.
8. Load the plates to the instrument, following the instructions on the instrument display. Order of plates start with the tip plate and ends with the sample plate. The purification process starts immediately after loading the sample plate to the instrument.

   Make sure that the plates are loaded in the correct 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/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 DNA	Incorrect storage of the sample material	- Sample should be harvested, stored and homogenized properly - Avoid repeated thawing and freezing
Low Purity / Inhibition	Ethanol in the eluted DNA	- Increase the evaporation time for Wash Buffer II
	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
	Polyphenol oxidation	- Add reducing agent to Lysis Buffer PL/VG 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.

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