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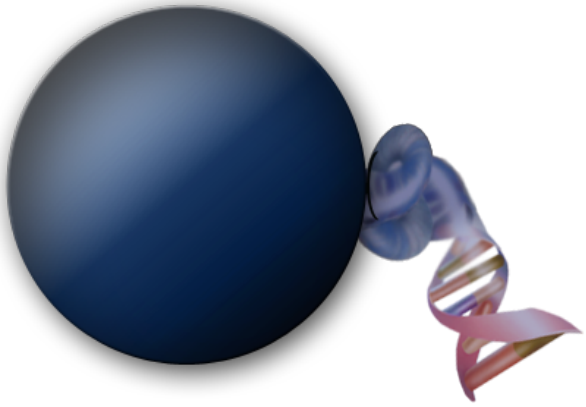
rQ MagSi-DNA Plant CLS

Art.No.

MDKT00260196PF



Product Manual

Revision 1.0 | 04-05-2023

Revision history		
Revision	**Release date**	**Remarks**
1.0	02/05/2023	Initial release

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Table of Contents

1. General Information

1.1 Intended Use

rQ 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 particle processors, such as the PurePrep 96 or KingFisher™ Flex Purification System. 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. rQ 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.

Processing time for DNA extraction from 96 plant lysates is about 30 minutes. The kit is provided ready-to-use, pre-filled in deepwell plates and requires no phenol/chloroform extraction or alcohol precipitation, and eliminates the need for repeated centrifugation, vacuum filtration or column separation. It allows safe handling of potentially infectious 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 rQ MagSi-DNA Plant CLS is directly usable in downstream analysis.

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. Nucleic acids are finally eluted in a volume of 150 µL Elution Buffer.

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. After removing the sealing foil from each reagent plate in the kit, the lysate is transferred to the rQ Plant CLS Binding Plate. All plates are loaded on the magnetic particle processor, and the instrument is started. When the run completed all plates are unloaded from the system, and the rQ Elution Plate containing the purified DNA is can directly be used for downstream applications.

2. Materials

2.1 Kit Contents

Component	96 preps MDKT00260196PF
Lysis Buffer VG	50 mL
Lysis Buffer PL	50 mL
rQ Plant CLS Binding Plate	1 plate containing 420 µL per well
96 well Tip-Comb	2 Units
rQ Wash Buffer I	1 plate containing 800 µL per well
rQ Wash Buffer II	2 plates containing 800 µL per well
rQ Elution Buffer	1 plate containing 150 µL per well
Product Manual	1 Unit(s)

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
Magnetic particle processor	PurePrep 96 System KingFisher™ Flex Purification System with 96 Deep-Well Head (ThermoFisher)
Tissue homogenizer	2010 Geno/Grinder® (SPEX SamplePrep)
Heating (Lysis)	Incubator or water bath (≥65°C)
Centrifuge	Depending on plate type used, recommended >6.000 x g
Plate for TipComb loading	PurePrep 96 Deepwell Plate or KingFisher 96 deep-well plate (re-used)

3. Kit usage

3.1 Storage Conditions

All components of the rQ 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

After opening all components included in the kit are ready-to-use. If there is any precipitate present in the buffers, warm up to 25-37°C to dissolve the precipitate.

3.3 Instrument protocol files

Please contact magtivio for the most recent PurePrep 96 protocol files or BindIt software method files. We provide the corresponding files for direct import to the PurePrep 96 Systems or uploading to the KingFisher™ magnetic particle processors through the BindIt software. Refer to the PurePrep user instructions or BindIt software manual regarding the import/upload procedure of the supplied files to the instrument.

3.4 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 rQ 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.

3.5 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 increased. 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. The rQ Elution Plate contains 150 µL Elution Buffer. If higher DNA concentrations are needed, buffer can be removed. However, a minimum working of 50 µL has to be maintained, and the protocol file must be adjusted.

3.6 Product use limitations

rQ 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 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.
4. Carefully peel off the seal from the **rQ Plant CLS Binding Plate**. Transfer **400 µL cleared lysate** to the **rQ Plant CLS Binding Plate**.
5. Carefully peel off the seal from all remaining plates.

Note: If there is any precipitate present in the buffers, first warm the prefilled plates to 25-37°C to dissolve the precipitate before use. Spin down briefly at low centrifugal force (<1000 x g) for ~30 seconds to collect condensation to the bottom.

4.2 Protocol for the PurePrep 96 System

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

2. Put all plates on the corresponding positions in the instrument (see table below) and place a Tip-Comb in the **rQ Plant CLS Binding Plate**. 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

Plate (position) in the protocol	Component(s)	Content(s)
1	PurePrep 96 Deepwell Plate (not provided) Tip-Comb	Empty, for loading Tip-Comb only
2	rQ Plant CLS Binding Plate Sample lysate	420 µL 400 µL (added by user)
3	rQ Wash Buffer I	800 µL
4	rQ Wash Buffer II	800 µL
5	rQ Wash Buffer II	800 µL
8	rQ Elution Buffer	150 µL

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

4. 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

1. Switch on the KingFisher Flex magnetic particle processor and select the protocol from the user defined protocols.

2. Start the protocol.

3. 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.

4. 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
Degraded DNA	Incorrect storage of the sample material	- Sample should be harvested, stored and homogenized properly - Avoid repeated thawing and freezing
Low Purity / Inhibition	Salt in the eluate (high adsorption at 230 nm)	- Make sure that wash supernatants are efficiently removed - rQ Wash Buffer plates should be stored and used at RT
	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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