January 2022

MagAttract® 96 DNA Plant Handbook

For efficient high-throughput isolation of DNA from plant tissue

Sample to Insight

QIAGEN

Contents

Kit Contents

MagAttract 96 DNA Plant Core Kit **Catalog no.** **No. of preps**	**(24)** **67163** **2304 (24 x 96)**
MagAttract Suspension G	100 ml
Buffer RB	2 x 200 ml
Buffer RLT*	4 x 220 ml
Buffer RPW*	4 x 125 ml
RNase A (100 mg/ml)	4 x 220 µl
Buffer AE	3 x 128 ml

* Contains harmful chemicals. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with bleach. See page 6 for detailed Safety Information.

Storage

All kit components, buffers, and RNase A stock solution can be stored at room temperature (15–25°C). After addition of RNase A and isopropanol, Buffer RPW is stable for 6 months when stored at 2–8°C.

Under these conditions, the components are stable for 6 months without showing any reduction in performance and quality.

Intended Use

The MagAttract 96 DNA Plant Core Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION 	Do not add bleach or acidic solutions directly to the sample preparation waste.

Buffers RPW and RLT contain guanidine hydrochloride/guanidine thiocyanate, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of MagAttract 96 DNA Plant Core Kit is tested against predetermined specifications to ensure consistent product quality.

Product Specifications

MagAttract 96 DNA Plant Core Kit	
Plant starting material: fresh/frozen	10–100 mg
Plant starting material: dried/lyophilized	10–30 mg
DNA yield*	1–15 µg
Final volume of eluate*	100 µl

* Depends on type of plant and preparation protocol used.

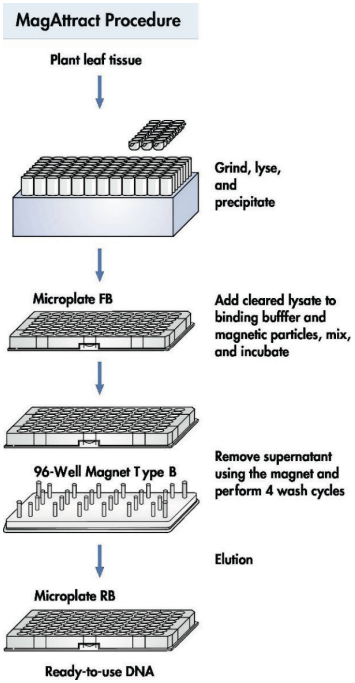
Recommended starting amounts and expected yields from different plants

Sample	Amount	Yield (µg DNA/mg tissue)	Total yield (µg)	Concentration (ng/µl)
Arabidopsis blossom and hull	3 pieces	0.46	4.6	57
Arabidopsis leaf	100 mg	0.05	5.0	62
Barley leaf	30 mg	0.15	4.5	56
Corn leaf	60 mg	0.20	12.0	150
Flax	40 mg	0.14	5.5	64
Rape (*Canola*)	40 mg	0.14	5.7	67
Rye leaf	30 mg	0.17	5.1	63
Ryegrass (Lolium spp.)	30 mg	0.13	4.0	47
Sunflower leaf	30 mg	0.16	4.8	60
Tobacco leaf	40 mg	–	1.3	
Tomato leaf	50 mg	0.15	7.5	93
Wheat leaf	30 mg	0.16	4.8	60

DNA has also been successfully purified from many other plants, including sugar beet (leaves and seeds), rose, *Lactuca serriola*, *Amaranthus*, maple, soy, cotton, rice, peanut, castor, gherkin, red gherkin, spinach, cauliflower, onion, white cabbage, carrot, red beet, bleach celery, and oat.

Introduction

The MagAttract 96 DNA Plant Core Kit combines the speed and efficiency of silica-based DNA purification with the convenience of magnetic particles, and is designed for fully automated high-throughput minipreparation of genomic, chloroplast, and mitochondrial DNA from plant tissue. MagAttract technology provides high-purity DNA, which is ready for use in downstream applications, such as PCR. This handbook contains a manual protocol, a general protocol for use of the MagAttract 96 DNA Plant procedure with robotic workstations/magnetic separation devices, and a specific protocol for use with the BioRobot® Plant Science Workstation.*



* QIAGEN robotic systems are not available in all countries; please inquire.

Principle and procedure

Fresh, frozen, or lyophilized starting material (10–100 mg) is mechanically disrupted to give a fine powder. The powder is resuspended in lysis buffer, carefully mixed, and then sedimented by a short centrifugation step.

The lysates are transferred to 96-well flat-bottom microplates before starting the MagAttract purification procedure. Genomic DNA selectively binds to the surface of MagAttract particles and is further purified by washing the magnetic particles with alcohol-containing buffers and ethanol. Pure genomic DNA is eluted from the particles with low-salt Buffer AE into a 96-well microplate and is ready for use in downstream applications.

Disruption of plant tissue

For disruption of plant tissue, optimal results are obtained using the TissueLyser together with the TissueLyser Adapter Set 2 x 96 and Tungsten Carbide Beads (stainless steel beads can also be used). The TissueLyser provides rapid and efficient disruption of 2 x 96 samples in 2–4 minutes.

Plant material and a 3 mm bead are added to each of 192 collection microtubes in two racks. The racks are fixed into the clamps on the TissueLyser using adapter plates and disrupted by two 1 minute high-speed (30 Hz) shaking steps. We recommend the use of tungsten carbide beads as these provide better and more reproducible results than chrome steel beads. For some samples, such as small seeds, 5 mm beads should be used for grinding to ensure production of a homogenous plant powder.

Either fresh or lyophilized plant tissue samples can be processed using the TissueLyser. Frozen material should be disrupted under freezing conditions using liquid nitrogen. Fresh material can also be disrupted in lysis buffer, but this may cause shearing of high-molecular-weight DNA. Lyophilized material should be disrupted without lysis buffer.

Centrifugation

The recommended speed for the centrifugation step in the protocol is 6000 x *g*, using the Centrifuge 4-16S or the Centrifuge 4-16KS equipped with the QIAGEN Plate Rotor 2 x 96. If these centrifuges are not available, or the recommended speed cannot be applied on the given centrifuge, centrifuge the plates at maximum speed. Increase the time of centrifugation if necessary.

Handling guidelines for the Magnet, 96-well, Type B

The QIAGEN Magnet, 96-well, Type B is designed for rapid, efficient, and convenient separation of MagAttract particles from solutions in 96-well microplates and round-well blocks. The magnet consists of an array of 24 magnetic NdFeB rods that fit between the wells of a microplate or round-well block. Each magnetic rod quickly attracts the particles in four adjacent wells to one side of each well and holds the particles in place while the buffer is removed. When the microplate or round-well block is removed from the magnet, the MagAttract particles are easily resuspended in buffer, allowing thorough washing and elution.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Equipment

- Equipment for disrupting plant tissue. We recommend the TissueLyser with the TissueLyser Adapter Set 2 x 96 and reusable 3 mm tungsten carbide or stainless steel beads for optimal disruption.
- Centrifuge 4-16S or 4-16KS with Plate Rotor 2 x 96
- Magnet for separation compatible with 96-well flat-bottom and round-bottom microplates (e.g., QIAGEN Magnet, 96-well, Type B, cat. no. 9012916)
- Multichannel pipet with 300 µl maximum capacity (for manual preparation)
- BioRobot Plant Science Workstation or other robotic workstation for automated processing

Consumables

- Reagent reservoirs for the multichannel pipet (for manual preparation)
- Collection Microtubes with Collection Microtube Caps (for disruption; cat. nos. 19560 and 19566 or 120008)
- Flat-bottom 96-well microplates (e.g., 96-Well Microplates FB, cat. no. 36985)
- Round-bottom 96-well microplates (e.g., 96-Well Microplates RB, cat. no. 19581)

Reagents

- Isopropanol (99–100%)
- Ethanol (96–100%)
- Liquid nitrogen

Protocol: Disruption of Plant Tissue and Preparation of Cleared Lysates

This protocol can be used for disruption of 192 (2 x 96) plant tissue samples using the TissueLyser (see “Disruption of plant tissue”, page 9). After addition of lysis buffer, cleared lysates are prepared by centrifugation.

Important: The optimal amount of starting material depends on the plant type and its state (fresh or lyophilized). We recommend using up to 100 mg fresh plant material, or up to 30 mg lyophilized material, and performing a preliminary experiment with different amounts of starting material.

Procedure

1. Place a plant tissue sample into each tube of two collection microtube racks.

   Keep the clear covers from the collection microtube racks for use in step 4. Normally 30 mg of starting material is sufficient. Do not use more than 50 mg (wet weight) unless preliminary experiments suggest that the optimal amount is higher.

2. Add one tungsten carbide or stainless steel bead to each collection microtube and seal the tubes with the caps supplied.

   Note that Buffer RLT may corrode tungsten carbide beads if samples are stored for more than 6 h.

3. Cool the collection microtubes in liquid nitrogen. Ensure that the microtubes remain tightly closed.

4. Place a clear cover (saved from step 1) over each rack of collection microtubes and knock the racks upside down against the bench 5 times to ensure that all tungsten carbide beads can move freely within the collection microtubes. Ensure that no liquid nitrogen remains but do not allow the leaf material to thaw. Remove the clear cover.

5. Sandwich each rack of collection microtubes between adapter plates and fix into the TissueLyser clamps as described in the TissueLyser instruction manual.

   Ensure that the microtubes are properly sealed with caps.

   Important: Two plate sandwiches must be clamped to the TissueLyser to provide balance. To process 96 samples or fewer, assemble a second plate sandwich using a rack of collection microtubes containing tungsten carbide beads but no samples or buffers, and fix it into the empty clamp.

6. Shake the samples for 1 min at 30 Hz.

7. Remove and dismantle the plate sandwiches. Ensure that the collection microtubes are tightly closed. Cool the collection microtube racks again in liquid nitrogen and then knock the racks against the bench 5 times to ensure that no tissue powder remains in the caps.

8. Reassemble the plate sandwiches so that the collection microtubes nearest the TissueLyser in steps 5 and 6 are now outermost. Reinsert the plate sandwiches into the TissueLyser.

   Rotating the racks of collection microtubes in this way ensures that all samples are thoroughly disrupted.

   Important: Merely rotating the entire plate sandwich so that the QIAGEN logos are upside down when reinserted into the TissueLyser is not sufficient since the samples that were outermost during the initial disruption will remain outermost in the second disruption step.

9. Shake the samples for 1 min at 30 Hz.

10. Carefully remove the caps from the collection microtubes and immediately pipet 300 µl Buffer RLT into each collection microtube.

11. Reseal the tubes with the caps and shake the entire rack in an upright position 20 times back and forth. Vortex the rack of collection microtubes upside down at full speed for 20 s.

12. Centrifuge the rack of collection microtubes for 5 min at 6000 x *g*.

13. Proceed with the relevant purification protocol.

Protocol: Manual DNA Purification

Things to do before starting

- Add 125 ml isopropanol and 1 vial RNase A (1 x 220 µl) to each bottle of Buffer RPW (125 ml) before use.
- Shake the bottle containing MagAttract Suspension G and vortex for 5 minutes (before first use) or 1 minute (before subsequent uses) to ensure that the magnetic particles are fully resuspended before use.

Procedure

1. Add 65 µl of Buffer RB to each well of a flat-bottom microplate or 1.5 ml microcentrifuge tube.

2. Add 20 µl of resuspended MagAttract Suspension G to each well of the 96-well flat-bottom microplate or into the 1.5 ml microcentrifuge tube.

   Note: Buffer RB and MagAttract Suspension G can be combined in appropriate proportions to make a master mix before starting the procedure. Add 85 µl of the master mix to each well of the 96-well flat-bottom microplate. Ensure that the MagAttract particles are fully resuspended.

3. Transfer 200 µl plant lysate supernatant into each well of the microplate or into the microcentrifuge tube, and mix by pipetting up and down several times.

4. Incubate at room temperature (15–25°C) for 5 min. Mix once during incubation.

   Note: Mixing can be done by pipetting using an 8-channel pipet. The pipet tips needed for this step can be reused if they are returned to the tip rack after use. To avoid cross-contamination, ensure that the same tip is always used for the same well.

   When using microcentrifuge tubes, samples can be mixed by vortexing.

5. Place the plate or microcentrifuge tube on the magnet and remove the supernatant after magnetic separation.

6. Wash the pelleted MagAttract particles by adding 200 µl Buffer RPW, resuspending the particles, placing the plate or tube on the magnet, and removing the supernatant.

   Note: Resuspension of the magnetic particles should be done very carefully since the efficiency of washing is directly related to how well the particles are resuspended. Resuspension can be performed by pipetting (see previous note) or by vortexing. If 96-well plates are vortexed, the initial mixing should be done very carefully to avoid spilling the magnetic particle suspension out of the wells and contaminating others. If this is too difficult with an initial volume of 200 µl, add 100 µl of Buffer RPW, vortex, and then add an additional 100 µl.

7. Wash the pelleted MagAttract particles by adding 200 µl ethanol (96–100%), resuspending the particles, placing the plate or tube on the magnet, and removing the supernatant.

8. Repeat step 7, aspirating as much ethanol as possible.

9. Dry the MagAttract particles for 5–10 min at room temperature (15–25°C).

10. Resuspend the MagAttract particles in 100 µl Buffer AE.

11. Incubate at room temperature for 5 min.

12. Place the plate or microcentrifuge tube on a magnet and transfer the DNA eluates to a clean 96-well round-bottom microplate or microcentrifuge tube.

Recommendations for Using MagAttract Technology with Robotic Systems

The following guidelines should be followed when using robotic liquid handling and processing platforms for the MagAttract 96 DNA Plant procedure, and as a starting point for optimization.

All automated protocols for the MagAttract 96 DNA Plant Core Kit start with cleared plant lysates (step 13 on page 14). Robotic platforms should be equipped with the following features:

- At least an 8-channel pipetting system; ideally a 96-channel system in addition to a 4- or 8-channel system
- Plate handling device ("robotic hand") to move plates
- Plate shaker with a shaking speed higher than 500 rpm for resuspension of magnetic particles
- Magnet for separation (e.g., QIAGEN Magnet, 96-well, Type B)
- Plate heating device to dry MagAttract particles (optional)

Since different robotic platform have different features and programming options, we can provide only general recommendations for the MagAttract 96 DNA Plant procedure. The main steps of the protocol are summarized below.

A fully automated protocol on the QIAGEN BioRobot Plant Science Workstation is also available (page **Error! Bookmark not defined.**).

Dispensing MagAttract Suspension G

MagAttract particles sediment if stored without agitation. To ensure a uniform distribution of particles, shake the magnetic particle suspension before dispensing.

To resuspend the MagAttract particles, we recommend pipetting 4 ml of MagAttract Suspension G into a 20 ml trough and shaking the trough for 90 seconds at 600 rpm on a plate shaker that is integrated in the worktable. The trough holder should have an "SBS-Standard" footprint for fitting into the plate shaker.

Resuspension of the pelleted MagAttract particles after magnetic separation

Some robotic devices are not capable of resuspending MagAttract particles by pipetting repeatedly after magnetic separation.

If this is the case, we recommend:

1. Addition of 100 µl Buffer RPW (wash buffer)
2. Resuspension of magnetic particles on a plate shaker (2 min at 800 rpm)
3. Adding an additional 100 µl buffer in each of the three wash steps to increase the wash efficiency

When using repeated pipetting to resuspend magnetic particles, the following guidelines may help:

1. Apply low-speed pipetting to loosen the pellet.
2. Apply high-speed pipetting for efficient homogenization of the particles once the pellet is loosened.

Removal of the supernatant after magnetic separation

Before elution, the MagAttract particles should be dried to remove the remaining ethanol.

This step can be done at room temperature (15–25°C), but the time for drying can be reduced and the reproducibility improved if the robotic workstation has an integrated heating device for microplates. We recommend drying the particles for 7 minutes at 50°C, although the drying time is dependent on the efficiency of the removal of the supernatant after the last wash cycle.

Note: The particles should not be overdried since this makes resuspension of the particles in elution buffer on an automated workstation very difficult.

An alternative to the drying step

The particles can be rinsed with distilled water while the microplate is on the magnet. The plate is kept on the magnet after removal of the supernatant from the last wash cycle to keep the particles fixed to the wall of the wells. Distilled water is then dispensed into the wells and is immediately aspirated. All pipetting is carried out at low speed.

Note: Rinsing the MagAttract particles with water while they are attracted to the magnet may slightly decrease the yield of DNA.

Protocol: Adaptation of MagAttract Technology to Robotic Systems

Some steps of the MagAttract 96 DNA Plant procedure can be automated using a standalone 96-channel pipetting device such as the BioRobot RapidPlate (protocol available upon request from QIAGEN Technical Services). Walkaway automation can be established on some workstations. This section provides general guidelines for establishing the MagAttract procedure on robotic systems.

Things to do before starting

- Add 125 ml isopropanol and 1 vial RNase A (1 x 220 µl) to each bottle of Buffer RPW (125 ml) before use.
- Shake the bottle containing MagAttract Suspension G and vortex for 5 minutes (before first use) or 1 minute (before subsequent uses) to ensure that the magnetic particles are fully resuspended before use.

Procedure

1. Add 65 µl of Buffer RB to each well of a 96-well flat-bottom microplate.
2. Add 20 µl of resuspended MagAttract Suspension G to each well of the 96-well flat-bottom microplate containing Buffer RB.

   Note: Buffer RB and MagAttract Suspension G can be combined in appropriate proportions to make a master mix before starting the procedure. Add 85 µl master mix to each well of the 96-well flat-bottom microplate. Ensure that the MagAttract particles are fully resuspended.
3. Carefully transfer 200 µl of each supernatant from the plant lysates to the 96-well flat-bottom microplate containing MagAttract Suspension G and Buffer RB. Mix the samples thoroughly by pipetting up and down several times.

4. Incubate the samples for 2 min at room temperature (15–25°C). Mix thoroughly by pipetting up and down and incubate for an additional 2 min at room temperature.

5. Place the flat-bottom microplate onto a suitable magnet, allow the MagAttract particles to separate for 20 s, and then remove the supernatant.

   Some robotic systems are capable of removing the supernatant slightly off-center of each well. This minimizes the danger of particle carryover.

6. Add 200 µl of Buffer RPW and resuspend the pelleted MagAttract particles thoroughly by pipetting up and down or by shaking.

7. Place the flat-bottom microplate onto the magnet, allow the MagAttract particles to separate for 20 s, and remove the supernatant.

8. Add 200 µl of ethanol (96–100%) and resuspend the MagAttract particles thoroughly by pipetting up and down or by shaking.

9. Place the flat-bottom microplate onto the magnet, allow the MagAttract particles to separate for 20 s, and remove the supernatant.

10. Repeat steps 8 and 9. During the final removal of the supernatant, try to aspirate as much of the ethanol as possible.

11. Dry the MagAttract particles for 5–10 min at room temperature.

12. Add 100 µl of Buffer AE to each well, resuspend the MagAttract particles thoroughly by pipetting up and down and/or vortexing, and incubate for 5 min at room temperature.

13. Place the flat-bottom microplate onto the magnet, allow the MagAttract particles to separate for 1 min, and transfer the supernatant to a clean 96-well round-bottom microplate.

    The DNA can be quantified directly or used for downstream reactions.

    Note: This step can be repeated if particle carryover occurs. We also recommend repeating it if the purified DNA is to be used in highly sensitive downstream applications.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page in our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		Comments and suggestions
Low or no DNA recovery		
a)	Low DNA content of the plant tissue	Increase the amount of starting material, but do not use more than 150 mg.
b)	Insufficient sample disruption	Ensure that the starting material is completely disrupted. See "Disruption of plant tissue", page 9.
c)	Insufficient sample lysis	Ensure that the starting material is resuspended thoroughly in Buffer RLT. Frozen samples should be resuspended immediately after disruption. Do not allow samples to thaw. If multiple Collection Microtube racks are to be processed, store the disrupted samples at –20°C before resuspending in Buffer RLT
d)	Incorrect binding conditions	Make sure that the correct amount of Buffer RB was added to the samples. **Note**: Some plants contain secondary metabolites, which may influence the binding of DNA to the MagAttract particles (see next point).
e)	Oils or other secondary metabolites may interfere with the binding process	In future preparations, try the protocol modifications for "difficult" plant materials suggested in Appendix A, page 26.
f)	MagAttract particles were not completely resuspended	Before starting the procedure, ensure that the MagAttract particles are fully resuspended. Vortex for at least 5 min before first use and for 1 min before subsequent uses.
g)	Buffer RPW did not contain isopropanol	Isopropanol must be added to Buffer RPW before use. Repeat procedure with correctly prepared Buffer RPW.
h)	The robotic workstation could not detect the elution buffer	The robotic workstation could have problems with liquid detection if distilled water is used for elution. Use Buffer AE for elution to prevent this problem.

	Comments and suggestions
Contamination of RNA in the eluate	
Buffer RPW did not contain RNase A	RNase A must be added to Buffer RPW before use. Repeat procedure with correctly prepared Buffer RPW. A low level of RNA contamination may not affect the results of quantitative PCR.
Magnetic particles in the eluate	
Magnetic particle carryover	Remove supernatant from MagAttract particles carefully, and from slightly off-center of each well. However, magnetic particles do not interfere with enzymatic reactions, such as PCR.
Overestimation of DNA yield upon spectrophotometric analysis	
a) RNA contamination in the eluate	Check that RNase A was added to Buffer RPW. Overestimation of yield can lead to failure of downstream applications
b) Insufficient washing of the MagAttract particles	Change the pipetting speeds and their duration, or increase the number of wash steps to improve the wash efficiency.
"Salt-peaks" at 200–240 nm in spectrophotometric scans	
Insufficient washing of the MagAttract particles	Change the pipetting speeds and their duration or the number of wash steps to improve the wash efficiency. However, salt carryover does not necessarily interfere with downstream applications
Green-, red-, or yellow-colored eluates	
Insufficient washing of the MagAttract particles	Chlorophyll or carotenoids can be efficiently removed by adding one additional wash step using ethanol or by reducing the amount of starting material in future preparations. Slight color in the eluate does not necessarily interfere with downstream applications.
Pipetting problems	
a) Lysate too viscous	In future preparations, reduce the amount of starting material and/or increase the volume of Buffer RLT to 400–600 µl. Use 200 µl of supernatant in the MagAttract 96 DNA Plant procedure.
b) Viscous and turbid lysates	If seeds are used as starting material, the crude lysate may be very viscous and turbid. This may result in low yields of DNA, problems with sample processing, and carryover of inhibitors. Generally, a turbid lysate indicates that too much plant material was used. For optimal results, reduce the amount of starting material. If it is not possible to use less material, we recommend increasing the volume of Buffer RLT to 400–600 µl. If the ground material is highly absorbent, large buffer volumes may be used Use 200 µl of supernatant in the MagAttract 96 DNA Plant procedure.

		Comments and suggestions
c)	After binding of the DNA to the particles, the particles clump and form large complexes, making liquid handling difficult	In future preparations, reduce the amount of starting material and/or increase the volume of Buffer RLT to 400–600 µl. Use 200 µl of supernatant in the MagAttract 96 DNA Plant procedure.
Variation of yields across the plate		
a)	Varying amount of starting material across the plate	Adjust the amounts of starting materials accordingly.
b)	Racks of collection microtubes not turned during disruption (when using TissueLyser)	It is essential to turn the racks of collection microtubes during disruption in the TissueLyser to ensure that all samples are evenly disrupted (see page 13, step 8).
c)	Non-uniform sample disruption when using alternative disruption methods	Ensure that all samples are uniformly disrupted.
DNA does not perform well in downstream experiments		
a)	Ethanol carryover	Aspirate as much of the ethanol in the final wash step as possible. Dry the particles before elution to remove residual ethanol.
b)	Insufficient/excess DNA used in downstream application	Optimize the amount of DNA used in the downstream application, if necessary. Downstream applications can be adversely affected by insufficient or excess DNA.
c)	Salt carryover	Change the pipetting speeds and their duration, or increase the number of wash steps to improve the wash efficiency.
d)	Eluate is slightly green or yellow	See “Green-, red-, or yellow-colored eluates”.

Appendix A: Protocol Modifications for "Difficult" Plant Materials

Some plant material used for DNA purification may yield low amounts of DNA and/or potential inhibitors of PCR may be co-purified. Carefully following the protocols in this handbook is important to optimize yields. If low yields or co-purification of inhibitors still occur, we recommend the following strategy.

Note: The automation protocols must be adapted to include the appropriate volume settings and worktable accessories.

Problem	Suggestion
Low yield of DNA	Replace Buffer RB (65 µl) with ethanol (225 µl). For some plant material, yields are higher if the binding conditions are modified by the addition of ethanol. Check whether your magnetic separation principle/device is suited for this option, because addition of alcohol affects the physical behavior of the magnetic particles. Note that the ethanol substitution for Buffer RB is not suitable for the automated BioRobot Plant Science protocol.
Inhibition of PCR	Replace Buffer RLT by Buffer AP1 (not included in the kit: contact QIAGEN Technical Services for details). Note that in many cases, the yield will be lower (depending on the sample source), but the performance in PCR may be better. Increase the volume of Buffer RLT used for lysis to up to 400–600 µl, but process only the recommended 200 µl of lysate for DNA purification. These recommendations can also be followed when using Buffer AP1. Polyphenolic compounds may be copurified with some plant materials and these can inhibit downstream reactions. Polyphenols can be removed by adding 33 mg/ml insoluble polyvinyl polypyrrolidone (PVPP) to Buffer RLT before use. PVPP forms complex hydrogen bonds with polyphenolic compounds, which are then separated from the DNA during the lysate centrifugation step. Prepare a small aliquot of Buffer RLT containing 33 mg/ml PVPP and thoroughly vortex the modified lysis buffer before use.
Low yields and inhibition of PCR	Replace Buffer RLT with Buffer AP1. Replace Buffer RB (65 µl) with ethanol (225 µl). Check whether your magnetic separation principle/device is suited for this option because addition of alcohol affects the physical behavior of the magnetic particles. Note that the ethanol substitution for Buffer RB is not suitable for the automated BioRobot Plant Science protocol.

Appendix B: Using 1 ml Round-Well Blocks for the MagAttract 96 DNA Plant Procedure

Round-well blocks with 1 ml wells can be used on some robotic workstations, allowing larger volumes of plant lysate to be processed. The protocol can be adapted to these plates, but note that standard 96-well microplates may provide better results on some workstations. The use of 1 ml round-well blocks may result in insufficient resuspension of the magnetic particles after magnetic separation.

The following recommendations for adaptation of the MagAttract 96 DNA Plant procedure to 1 ml round-well blocks are provided as a guide:

- Resuspend the disrupted plant material in 500 µl Buffer RLT
- Add 400 µl cleared plant lysate to 130 µl Buffer RB and 20 µl MagAttract Suspension G
- Use 200–400 µl of Buffer RPW for the first washing step, followed by 200–400 µl ethanol for the second and third washing steps

The volume of wash buffer required is dependent on the speed of the shaking platform used to resuspend the MagAttract particles.

Appendix C: Determination of DNA Concentration and Yield

DNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. For greatest accuracy, absorbance readings at 260 nm should be between 0.1 and 1.0. Sample dilution should be adjusted accordingly: for example, an eluate with an expected DNA concentration of 25–50 ng/µl (A_{260} = 0.5–1) should not be diluted more than fourfold for spectrophotometry. An absorbance of 1 at 260 nm corresponds to 50 µg DNA per ml. This relationship is only valid for measurements made in water; therefore, samples should be diluted in water. Use water to zero the spectrophotometer.

We recommend measuring the absorbance at 260 nm of the purified MagAttract 96 DNA Plant samples using a fourfold dilution. DNA yields from different plant species depend greatly on genome size and ploidy and also on the age and growth state of the plant.

Appendix D: Recovery and Cleaning of Tungsten Carbide Beads or Stainless Steel Beads

Tungsten carbide beads are designed for repeated use. Used tungsten carbide beads can be recovered from cell-debris pellets and cleaned using the procedure below.

1. Seal the collection microtubes with caps. Place a clear cover (saved from step 1 of the MagAttract 96 DNA Plant procedure, page 12) over each rack of collection microtubes and knock the racks upside down against the bench 5 times to free the tungsten carbide beads from the surrounding material.
2. Empty the contents of the microtubes into a fine sieve and rinse the beads thoroughly with water.
3. Incubate beads in 0.4 M HCl for 1 min at room temperature (15–25°C) to degrade any DNA and avoid cross-contamination in future preparations.
4. Rinse beads thoroughly with distilled water to remove the HCl.

Appendix E: Cleaning of S-Blocks

To avoid cross-contamination, after each use rinse the S-Blocks thoroughly in tap water, incubate for 1 minute at room temperature in 0.4 M HCl, empty, and wash thoroughly with distilled water. Used S-Blocks can also be autoclaved after washing. Additional S-Blocks can be ordered separately (see Ordering Information, page 30).

Ordering Information

Product	Contents	Cat. no.
MagAttract 96 DNA Plant Core Kit (24)	MagAttract Suspension G and buffers for 24 x 96 minipreps	67163
Accessories		
Collection Microtubes (racked)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
Collection Microtube Caps	Nonsterile polypropylene caps for collection microtubes (1.2 ml), 960 in strips of 8	19566
Elution Microtubes RS (24 x 96)	Elution Microtubes RS (EMTR) for use with QIAcube HT: 24 x 96 Elution Microtubes in racks of 96; includes strip caps	120008
96-Well Microplates FB (24)	96-well microplates with flat-bottom wells, 24 per case, for use with the 96-Well Magnet	36985
96-Well Microplates RB (24)	96-well microplates with round-bottom wells plus lids, 24 per case	19581
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
S-Blocks	96-well blocks with 2.2 ml wells, 24 per case	19585

Magnet, 96-Well, Type B	Magnet for separating magnetic beads in wells of 96-well microplates	9012916
TissueLyser 24	Universal laboratory mixer mill, for 24 samples	Inquire
TissueLyser 96	Universal laboratory mixer mill, for 96 samples	Inquire

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Document Revision History

Date	Changes
07/2015	Initial revision
01/2022	Updated the Kit Contents and Storage sections. Corrected a typographical error: from “MagAttract Suspension A” to “MagAttract Suspension G”. Removed the section “Protocol: DNA Purification Using the MagAttract 96 DNA Plant Core Kit and the BioRobot Plant Science Workstation”. Updated centrifuge names. Updated the content of Ordering Information section.

Notes

Notes

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