February 2016

BioSprint® DNA Plant Handbook

For purification of total DNA from plant samples using BioSprint workstations

Sample to Insight

QIAGEN

Contents

Kit Contents

BioSprint 15 DNA Plant Kit	**(360)**
Catalog no.	**941517**
Number of preps	**360**
Buffer RLT*	2 x 45 ml
RNase A	220 µl
MagAttract® Suspension G†	13 ml
Buffer RPW* (concentrate)	125 ml
5-Rod Cover	72
5-Tube Strip (1 ml)	360
Quick-Start Protocol	1

BioSprint 96 DNA Plant Kit	**(576)**	**(1536)**
Catalog no.	**941557**	**941558**
Number of preps	**576**	**1536**
Buffer RLT*	220 ml	2 x 220 ml
RNase A	2 x 220 µl	4 x 220 µl
MagAttract Suspension G†	13 ml	3 x 13 ml
Buffer RPW* (concentrate)	2 x 125 ml	4 x 125 ml
Large 96-Rod Cover	6	16
96-Well Microplate MP	8	18
S-Block	30	80
Quick-Start Protocol	1	1

* **CAUTION:** Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 4 for safety information.

† **CAUTION:** Contains sodium azide as a preservative.

Storage

All kit components can be stored dry at room temperature (15–25°C) for up to 1 year without showing any reduction in performance. After addition of RNase A and isopropanol, Buffer RPW should be stored at 2–8°C and is stable for 6 months.

Intended Use

BioSprint DNA Plant Kits are intended for purification of total DNA from plant samples using BioSprint workstations. BioSprint DNA Plant Kits are intended for molecular biology applications. These products are 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.

Buffer RLT contains guanidine thiocyanate and Buffer RPW contains guanidine hydrochloride, which can form highly reactive compounds if combined with bleach.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled 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 BioSprint DNA Plant Kits is tested against predetermined specifications to ensure consistent product quality.

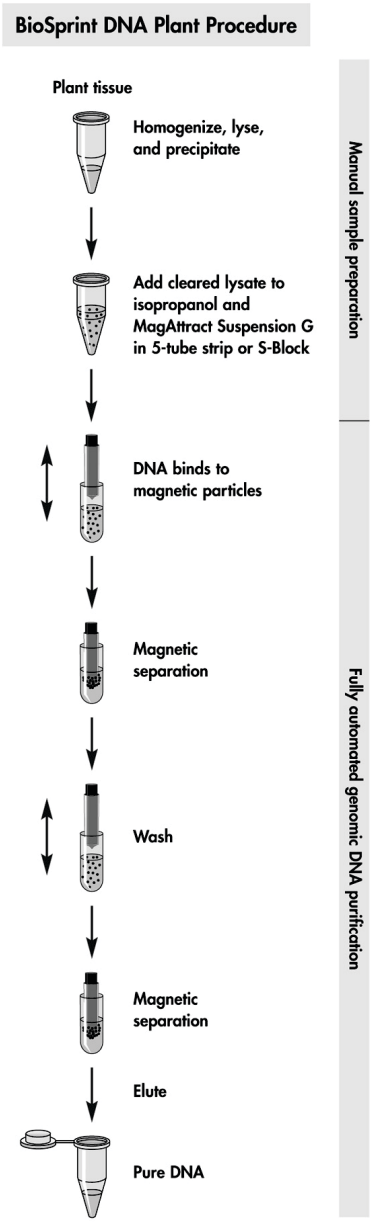
Introduction

BioSprint DNA Plant Kits are designed for purification of total DNA (i.e., genomic, chloroplast and mitochondrial DNA) from plant material, such as leaves and seeds, using BioSprint workstations. BioSprint DNA Plant Kits provide high-quality DNA that is free of protein, nucleases, and other contaminants or inhibitors. The DNA is suitable for direct use in downstream applications, such as amplification or other enzymatic reactions.

Principle and procedure

BioSprint DNA Plant Kits use MagAttract magnetic-particle technology for DNA purification. MagAttract technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (see flowchart). Fresh, frozen, or lyophilized starting material 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 cleared lysates are either transferred to a 5-well strip for processing on the BioSprint 15 or to an S-Block for processing on the BioSprint 96.

DNA binds to the silica surface of MagAttract magnetic particles in the presence of a chaotropic salt. DNA bound to the magnetic particles is then efficiently washed with alcohol-containing buffers and ethanol, followed by a rapid rinse with distilled water or an air drying step, which considerably improves the purity of the DNA. High-quality DNA is eluted in water or low-salt buffer.


BioSprint DNA Plant Procedure
Plant tissue
Homogenize, lyse, and precipitate
Add cleared lysate to isopropanol and MagAttract Suspension G in 5-tube strip or S-Block
DNA binds to magnetic particles
Magnetic separation
Wash
Magnetic separation
Elute
Pure DNA
Manual sample preparation
Fully automated genomic DNA purification

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.

For all protocols:

- Pipets and disposable pipet tips with aerosol barriers (20–1000 µl)
- Isopropanol(100%)
- Ethanol (96–100%)*
- Distilled water or low-salt buffer, such as Buffer AE (cat. no. 19077), for elution of DNA
- Disposable gloves
- Vortexer
- Liquid nitrogen
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean worktable

For the BioSprint 15 DNA Plant protocol

- BioSprint 15 workstation
- Equipment for disrupting plant tissue. We recommend the TissueLyser II with the TissueLyser Adapter Set 2 x 24 and 3 mm Tungsten Carbide or 5 mm Stainless Steel Beads for optimal disruption (see page 29 for ordering information).
- Safe-lock microcentrifuge tubes (2 ml) (for homogenization using the TissueLyser II) and tubes for storage of purified DNA
- Microcentrifuge capable of attaining 6000 x *g* (with rotor for 2 ml tubes)
- Multidispenser/multichannel pipettor

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

For the BioSprint 96 DNA Plant protocol

- BioSprint 96 workstation
- Magnetic head for use with large 96-rod covers (supplied with the BioSprint 96)
- Equipment for disrupting plant tissue. We recommend the TissueLyser II with the TissueLyser Adapter Set 2 x 96 and 3 mm Tungsten Carbide or 5 mm Stainless Steel Beads for optimal disruption (see page 29 for ordering information).
- Collection Microtubes (racked, 10 x 96) (cat. no. 19560) with Collection Microtube Caps (120 x 8) (cat. no. 19566) (for homogenization using the TissueLyser II)
- Multichannel pipettor and disposable pipet tips with aerosol barriers
- Troughs for use with a multichannel pipettor
- Distilled water for washing the magnetic particles
- Tween® 20
- Centrifuge 4-16S or 4-16KS with Plate Rotor 2 x 96 (see page 29 for ordering information)

Important Notes

Collection and storage of starting material

After harvesting, plant tissue should be frozen in liquid nitrogen. It can then be stored at –80°C for later processing. Ground tissue powder can also be stored at –80°C. Alternatively, tissue can be freeze-dried/lyophilized after harvesting to allow storage at room temperature (15–25°C).

If possible, it is preferable to collect young materials (e.g., leaves, needles) since they contain more cells per weight and therefore result in higher yields of DNA. In addition, young leaves and needles contain less polysaccharides and polyphenolics and are therefore easier to handle.

Disruption of plant tissue

Complete and quick disruption of starting material is essential to ensure high DNA yields and to avoid DNA degradation. Optimal results are obtained using the TissueLyser II together with either the TissueLyser Adapter Set 2 x 24 or the TissueLyser Adapter Set 2 x 96 (see page 29 for ordering information). The TissueLyser II provides rapid and efficient disruption of up to 96 samples in parallel in 2–5 minutes.

Plant material and a 3 mm tungsten carbide bead are added to 2 ml safe-lock microcentrifuge tubes (when using the TissueLyser Adapter Set 2 x 24) or collection microtubes (when using the TissueLyser Adapter Set 2 x 96) in two racks. The racks are fixed into the clamps on the TissueLyser II 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 stainless steel beads. For some samples, such as small seeds, 5 mm stainless steel beads should be used for

grinding to ensure production of a homogenous plant powder. If necessary, two 3 mm tungsten carbide beads can be used per tube.

Fresh, frozen, or lyophilized plant tissue samples can be processed using the TissueLyser II. Fresh or frozen samples can be disrupted after freezing in liquid nitrogen without lysis buffer. Alternatively, fresh material can be directly disrupted in lysis buffer without using liquid nitrogen, but this may cause shearing of high-molecular-weight DNA. Lyophilized material should be disrupted without lysis buffer at ambient temperature. We do not recommend disrupting frozen material in lysis buffer as this results in low yields and degraded DNA. Disruption of samples in lysis buffer yields DNA that is suitable for PCR, while disruption of samples in liquid nitrogen yields DNA of a higher molecular weight.

Plant tissue can also be manually disrupted by grinding under liquid nitrogen using a mortar and pestle. Optimization of the manual disruption method may be required to ensure maximum DNA yield and quality.

Centrifugation

The recommended speed for the centrifugation step in the protocol is 6000 x *g*. The Centrifuge 4-16S or the Centrifuge 4-16KS equipped with the QIAGEN Plate Rotor 2 x 96 can be used if processing samples in collection microtubes (see page 29 for ordering information). 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.

Yield and quality of purified DNA

DNA yields depend on the sample type, the sample collection method used, and the method of tissue disruption. The BioSprint DNA Plant procedure is optimized for 30–50 mg fresh plant material or up to 30 mg lyophilized material. With some plant types, up to 100 mg of fresh plant material can be used. Exceeding the recommended maximum amount of starting material will result in inefficient lysis, resulting in low DNA yield and purity. DNA yields vary depending on genome size, ploidy, and age of sample. Yields typically range from 5–20 µg per 30–50 mg of wet-weight sample. We strongly recommend performing a preliminary experiment with different amounts of starting material.

Elution in smaller volumes increases the final DNA concentration in the eluate. We recommend using an elution volume appropriate for the intended downstream application.

Preparing reagents

Buffer RPW

Buffer RPW is supplied as a concentrate. Before using for the first time, add 125 ml isopropanol and 1 vial RNase A (1 x 220 µl) to each bottle of Buffer RPW as described on the bottle label. Store reconstituted Buffer RPW at 2–8°C.

MagAttract suspension G

To ensure that the magnetic silica particles are fully resuspended, MagAttract Suspension G must be shaken and vortexed before use. Before first use, shake the vial or bottle, and vortex for 3 minutes. Before subsequent uses, shake the bottle and vortex for 1 minute.

Distilled water

In the BioSprint 96 DNA Plant procedure, magnetic particles are briefly washed with distilled water to remove residual ethanol from the previous wash step. Tween 20 must be added to the distilled water to a final concentration of 0.02% (v/v) (e.g., add 6 µl and 50 µl Tween 20 to 30 ml and 250 ml distilled water, respectively).

Note: Distilled water containing Tween 20 should only be used to wash the magnetic particles. For elution of DNA, use distilled water that does not contain Tween 20.

Quantification of DNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified DNA, but should not affect downstream applications. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. See Appendix, page 27, for more information.

Protocol: Purification of DNA from Plant Tissue Using the BioSprint 15

This protocol is for purification of genomic, chloroplast, and mitochondrial DNA from plant material using the BioSprint 15 workstation and the BioSprint 15 DNA Plant Kit. We recommend using 30–50 mg fresh plant material or up to 30 mg lyophilized material.

Important points before starting

- The optimal amount of starting material depends on the plant type and its state (fresh or lyophilized). We recommend using 30–50 mg fresh plant material or up to 30 mg lyophilized material. In some cases, up to 100 mg fresh plant material can be used. We strongly recommend performing a preliminary experiment with different amounts of starting material.
- Ensure that you are familiar with operating the TissueLyser II and the BioSprint 15. Refer to the *TissueLyser II Handbook* and the *BioSprint 15 User Manual* for operating instructions.

Things to do before starting

- Ensure that Buffer RPW has been prepared as described on page 12.

Disruption of plant tissue using the TissueLyser II

1. Place a plant tissue sample into a 2 ml safe-lock microcentrifuge tube.

   If processing fresh or frozen plant tissue, 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. Up to 30 mg of lyophilized material may be used.
2. Add one 3 mm tungsten carbide bead to each 2 ml safe-lock microcentrifuge tube and close the lid. If using fresh or frozen plant material, proceed to step 3. If using lyophilized plant material, proceed directly to step 4.

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

For some samples, such as small seeds, one 5 mm stainless steel bead can be used or two 3 mm tungsten carbide beads.

3. Cool the 2 ml safe-lock microcentrifuge tubes in liquid nitrogen for 30 s. Ensure that the tubes remain tightly closed.
4. Place the 2 ml safe-lock microcentrifuge tubes into the TissueLyser Adapter Set 2 x 24, and knock the assembled adapter set against the bench 5 times to ensure that all tungsten carbide beads can move freely within the tubes. If processing frozen plant material, ensure that no liquid nitrogen remains, but do not allow the plant material to thaw.
5. Fix the adapter set into the clamps of the TissueLyser II as described in the *TissueLyser II Handbook*, and homogenize the samples for 1 min at 30 Hz.

   Important: For optimal operation, the TissueLyser II should always be balanced. To process 24 or fewer samples, assemble a second adapter set with 2 ml safe-lock microcentrifuge tubes containing tungsten carbide beads but no samples or buffers, and fix it into the empty clamp.
6. Disassemble the adaptor set, and remove the 2 ml safe-lock microcentrifuge tubes. If using lyophilized plant material, proceed directly to step 7. If using fresh or frozen plant material, cool the samples in liquid nitrogen for 30 s.
7. Place the 2 ml safe-lock microcentrifuge tubes into the adapter set, reversing the order of the tubes within the adaptor set. Knock the reassembled adapter set against the bench 5 times to ensure that all tungsten carbide beads can move freely within the tubes.

   To prevent variation in sample homogenization, the adaptor set should be removed from the TissueLyser II and disassembled after the first disruption step. For the second disruption step, the adaptor set should be reassembled so that the microcentrifuge tube order is reversed. Rotating the microcentrifuge tubes in this way ensures that all samples are thoroughly and equally disrupted.

Note: The majority of plant tissue is ground to a fine powder after two disruption steps, however, for some materials one disruption step may be sufficient. Other tissues, such as seeds and roots, may need three disruption steps. Optimization of the disruption procedure may be required for some plant material.

8. Fix the adapter set into the clamps of the TissueLyser II, and homogenize the samples for 1 min at 30 Hz.
9. Knock the adapter set against the bench 5 times to ensure that no tissue powder remains in the lids of the tubes. Disassemble the adapter set, and carefully open the lid of each 2 ml safe-lock microcentrifuge tube. Immediately pipet 300 µl Buffer RLT into each tube.

   Note: Buffer RLT may corrode tungsten carbide beads if samples are stored for more than 6 hours.
10. Close the lid of each 2 ml safe-lock microcentrifuge tube and vortex vigorously.
11. Centrifuge the 2 ml safe-lock microcentrifuge tubes at 6000 x *g* for 5 min at room temperature (15–25°C).
12. Proceed with "Purification of DNA using the BioSprint 15", below.

Purification of DNA using the BioSprint 15

1. Switch on the BioSprint 15 at the power switch.
2. Open the front door of the BioSprint 15 and slide out the tube strip tray.
3. Load up to fifteen 5-tube strips into the tube strip tray. One 5-tube strip is used per sample.

   If loading five 5-tube strips or fewer, we recommend loading them as a single column. If loading ten 5-tube strips or fewer, we recommend loading them as 2 columns.

   Load the 5-tube strips in the tube strip tray so that the tab of each 5-tube strip faces to the left. Make sure that the 5-tube strips are fully inserted into the tray and are not skewed.

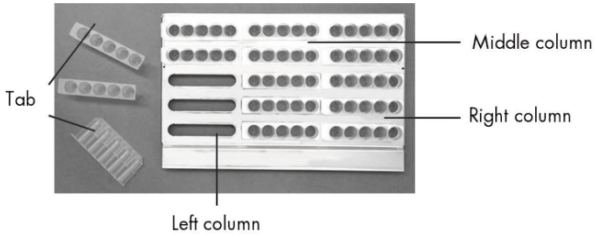


Figure 1. Correct loading of 5-tube strips in the tube strip tray.

4. Add reagents into each 5-tube strip according to Table 1.

   Note: Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 min before first use and for 1 min before subsequent uses.

Table 1. Reagents to add to 5-tube strips

Well	Regent	Volume to add)
1	Isopropanol	200 µl
1	MagAttract Suspension G	20 µl
2	Buffer RPW	500 µl
3	Ethanol (96–100%)	500 µl
4	Ethanol (96–100%)	500 µl
5	Distilled water or low-salt buffer	200 µl

Note: Well 1 is at the left of the 5-tube strip; well 5 is at the right.

5. Transfer 200 µl cleared plant lysate into well 1 of the 5-tube strip.
6. Load up to three 5-rod covers into the rod cover slots (Figure 2 and Figure 3). There must always be a 5-rod cover above a column of 5-tube strips.

   Note: If necessary, remove the tube strip tray to allow easier loading of the 5-rod covers.

   Insert a 5-rod cover into a rod cover slot so that the short tab faces inward and the long tab faces outward. 5-rod covers must be inserted so that they click into place.

Note: Do not push 5-rod covers further after they click into place or an instrument crash will occur.

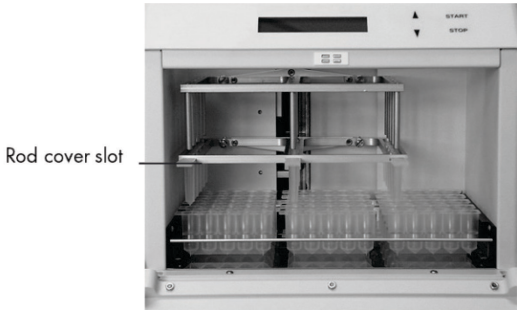


Figure 2. Rod cover slot.

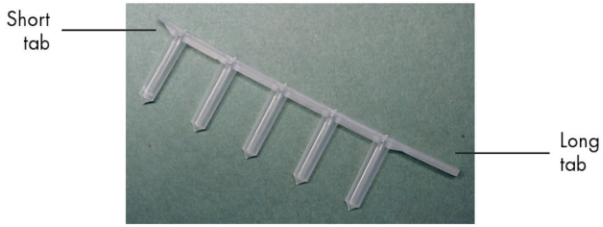


Figure 3. Tabs of the 5-rod cover.

7. Slide the tube strip tray fully into the BioSprint 15.
8. Close the front door of the BioSprint 15.

   Closing the front and top doors protects the samples from contamination.
9. Select the protocol "BS15 DNA Plant" using the ▲ and ▼ keys on the BioSprint 15 workstation. Press "Start" to start the protocol run.

   Warning: Avoid contact with moving parts during operation of the BioSprint 15. See the *BioSprint 15 User Manual* for safety information.

10. After the protocol run ends, press "Stop" and slide out the tube strip tray. Transfer the eluted DNA from well 5 of each 5-tube strip to other tubes for long-term storage.

    Note: Well 5 is at the right of the 5-tube strip.

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to clean tubes (see Appendix, page 27).

11. Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.

    Note: See page 4 for safety information.

12. Switch off the BioSprint 15 at the power switch.

13. Wipe the surface of the tube strip tray and adjacent surfaces with a soft cloth or tissue moistened with distilled water or a mild detergent solution. If infectious agents are spilt onto the tube strip tray, clean using 70% ethanol or other disinfectant.

    Note: Do not use bleach as disinfectant. See page 4 for safety information.

Protocol: Purification of DNA from Plant Tissue Using the BioSprint 96

This protocol is for purification of genomic, chloroplast, and mitochondrial DNA from plant material using the BioSprint 96 workstation and the BioSprint 96 DNA Plant Kit. We recommend using 30–50 mg fresh plant material or up to 30 mg lyophilized material.

Important points before starting

- The optimal amount of starting material depends on the plant type and its state (fresh or lyophilized). We recommend using 30–50 mg fresh plant material or up to 30 mg lyophilized material. In some cases, up to 100 mg fresh plant material can be used. We strongly recommend performing a preliminary experiment with different amounts of starting material.
- Ensure that you are familiar with operating the TissueLyser II and the BioSprint 96. Refer to the *TissueLyser II Handbook* and the *BioSprint 96 User Manual* for operating instructions.

Things to do before starting

- Ensure that Buffer RPW has been prepared as described on page 12.
- 96-rod covers are supplied either in packs of two, or as a pack of one inserted into an S-Block. If using a new pack of two, store the second 96-rod cover on another plate. It is important that the 96-rod cover does not become bent.

Disruption of plant tissue using the TissueLyser II

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. If processing fresh or frozen plant tissue, 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. Up to 30 mg of lyophilized material may be used.

2. Add one 3 mm tungsten carbide bead to each collection microtube, and seal the tubes with the caps supplied. If using fresh or frozen plant material, proceed to step 3. If using lyophilized plant material, proceed directly to step 4.

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

   For some samples, such as small seeds, one 5 mm stainless steel bead can be used or two 3 mm tungsten carbide beads.

3. Cool the collection microtubes in liquid nitrogen for 30 s. Ensure that the collection 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. If processing frozen plant material, ensure that no liquid nitrogen remains, but do not allow the plant material to thaw. Remove the clear cover.

5. Place each rack of collection microtubes between the adapter plates of the TissueLyser Adapter Set 2 x 96, and fix into the TissueLyser II clamps as described in the *TissueLyser II Handbook*. Ensure that the collection microtubes are properly sealed with caps.

   Important: Two plate sandwiches must be clamped to the TissueLyser II to provide balance. To process 96 or fewer samples, 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. Homogenize the samples for 1 min at 30 Hz.

7. Remove and dismantle the plate sandwiches. Ensure that the collection microtubes are tightly closed. If using lyophilized plant material, proceed directly to step 8. If using fresh or frozen plant material, cool the collection microtubes again in liquid nitrogen for 30 s.

8. Knock the racks against the bench 5 times to ensure that the tungsten carbide beads can move freely within the collection microtubes. Reassemble the plate sandwiches so that the collection microtubes nearest the TissueLyser II in steps 5 and 6 are now outermost. Reinsert the plate sandwiches into the TissueLyser II clamps.

   Rotating the racks of collection microtubes 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 II is not sufficient since the samples that were outermost during the initial disruption will remain outermost in the second disruption step.

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

10. Remove and dismantle the plate sandwiches. Knock the racks against the bench 5 times to ensure that no tissue powder remains in the caps. Carefully remove the caps and immediately pipet 300 µl Buffer RLT into each collection microtube.

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

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

12. Centrifuge the rack at 6000 x *g* for 5 min at room temperature (15–25°C).

13. Proceed with "Purification of DNA using the BioSprint 96", below.

Purification of DNA using the BioSprint 96

1. Prepare four S-Blocks (slots 2-5) and two 96-well microplates (slots 6 and 7) according to Table 2. The S-Blocks and microplates are loaded onto the worktable in step 8.

   In each plate or block, the number of wells to be filled with buffer should match the number of samples to be processed (e.g., if processing 48 samples, fill 48 wells per plate or block). Ensure that buffers are added to the same positions in each plate or block (e.g., if processing 48 samples, fill wells A1-H1 to A6-H6 of each plate or block).

   Note: For elution of DNA, use distilled water that does not contain Tween 20.

Table 2. Preparation of S-Blocks and microplates

Slot	Message when loading	Plate/block	To add	Volume to add per well
7	Load Rod Cover	96-well microplate MP	Large 96-well rod cover	–
6	Load Elution	96-well microplate MP	Distilled water or low-salt buffer	200 µl
5	Load Wash 4	S-Block	Distilled water*	500 µl
4	Load Wash 3	S-Block	Ethanol (96–100%)	500 µl
3	Load Wash 2	S-Block	Ethanol (96–100%)	500 µl
2	Load Wash 1	S-Block	Buffer RPW	500 µl
1	Load Lysate	S-Block	Lysate†	420 µl

* Contains 0.02% (v/v) Tween 20.

† Added in steps 2, 3 and 4: includes volume of cleared plant lysate, isopropanol and MagAttract Suspension G.

2. Transfer 200 µl cleared plant lysate into each well of an S-Block.
3. Add 200 µl isopropanol to each sample in the S-Block.
4. Add 20 µl MagAttract Suspension G to each sample in the S-Block.

   Note: Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 min before first use and for 1 min before subsequent uses.
5. Switch on the BioSprint 96 at the power switch.
6. Slide open the front door of the protective cover.
7. Select the protocol "BS96 DNA Plant" using the ▲ and ▼ keys on the BioSprint 96 workstation. Press "Start" to start the protocol run.
8. The LCD displays a message asking you to load slot 7 of the worktable with the 96-rod cover (Table 2, page 23). After loading slot 7, press "Start". The worktable rotates and a new message appears, asking you to load slot 6 with the elution plate. Load slot 6 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.

   Note: Each slot is labeled with a number. Load each 96-well plate or S-Block so that well A1 is aligned with the slot's label (i.e., well A1 faces inward).

9. Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.

   Warning: Avoid contact with moving parts during operation of the BioSprint 96. See the *BioSprint 96 User Manual* for safety information.

10. Press "Start" to start sample processing.

11. After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press "Start" after removing each plate or block. The first item to be removed contains the purified samples.

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, the microplate containing eluates should first be placed in a suitable magnet and the eluates transferred to a clean microplate (see Appendix, page 27).

12. Press "Stop" after all plates and blocks are removed.

13. Discard the used plates, blocks, and 96-rod cover according to your local safety regulations.

    Note: See page 4 for safety information.

14. Switch off the BioSprint 96 at the power switch.

15. Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.

    Note: Do not use bleach as disinfectant. See page 4 for safety information.

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 at 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 and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Low DNA yield	
a) Inefficient cell lysis due to insufficient homogenization/ disruption	Ensure that the starting material is completely disrupted. See "Disruption of plant tissue", page 10. Increase the homogenization time with the TissueLyser II and make sure the sample is properly frozen in liquid nitrogen.
b) Low DNA content of the plant tissue	Increase the amount of starting material up to a maximum of 100 mg. For plants with a small genome (e.g., *Arabidopsis thaliana*), increase the amount of starting material up to a maximum of 150 mg.
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 –30 to –15°C before resuspending in Buffer RLT.
d) No isopropanol added to the lysate before adding MagAttract Suspension G	Repeat the DNA purification procedure with a new sample.
e) MagAttract Suspension G not completely resuspended	Before starting the procedure, ensure that the MagAttract Suspension G is fully resuspended. Vortex for at least 3 minutes before first use and for 1 minute before subsequent uses.
f) Buffer RPW prepared incorrectly	Ensure that the correct volumes of isopropanol and RNase A were added to Buffer RPW. Repeat the DNA purification procedure with a new sample.

		Comments and suggestions
RNA contamination in the eluate		
	Buffer RPW missing 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.
DNA does not perform well in downstream applications		
	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.
A_{260}/A_{280} ratio for purified DNA is low		
a)	Inefficient cell lysis due to insufficient homogenizing of plant material	Increase the homogenization time with the TissueLyser II, and make sure the sample is properly frozen in liquid nitrogen.
b)	No isopropanol added to the lysate before adding MagAttract Suspension G	Repeat the DNA purification procedure with a new sample.
c)	MagAttract Suspension G not completely resuspended	Before starting the procedure, ensure that the MagAttract Suspension G is fully resuspended. Vortex for at least 3 minutes before first use and for 1 minute before subsequent uses.
d)	Buffer RPW prepared incorrectly	Ensure that the correct volumes of isopropanol and RNase A were added to Buffer RPW. Repeat the DNA purification procedure with a new sample.
e)	Absorbance reading at 320 nm not subtracted from the absorbance readings at 260 nm and 280 nm	To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see Appendix, page 27).

Appendix: Handling, Quantitation and Determination of Purity of DNA

Storage of DNA

Purified DNA may be stored at 2–8°C for 24 hours or at –30 to –15°C for longer periods.

Minimizing magnetic particle carryover in the DNA

If the purified DNA is to be analyzed by real-time PCR, any trace amounts of magnetic particles should be minimized using a magnet.

Transfer the eluates to 1.5 ml microcentrifuge tubes. Apply the tubes to a suitable magnet (e.g., QIAGEN 12-Tube Magnet) for 10 minutes, and carefully remove the supernatants. Alternatively, transfer the eluates to a flat-bottom microplate (e.g., QIAGEN 96-Well Microplate FB). Apply the microplate to a suitable magnet (e.g., QIAGEN 96-Well Magnet Type A) for 10 minutes, and carefully remove the supernatants.

If a suitable magnet is not available, transfer the eluates to microcentrifuge tubes, centrifuge for 1 minute at full speed to pellet any remaining magnetic particles, and carefully remove the supernatants.

Quantification and determination of purity of DNA

The concentration of DNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 nm corresponds to 50 µg of DNA per milliliter (A_{260} = 1 → 50 µg/ml). Use a low-salt buffer of neutral pH (e.g., 10 mM Tris·Cl,* pH 7) to dilute DNA samples and to calibrate the spectrophotometer.

The ratio between the absorbance values at 260 nm and 280 nm gives an estimate of DNA purity. For accurate results, use a slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5) to dilute DNA samples and to calibrate the spectrophotometer. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9.

Carryover of magnetic particles in the eluates may affect the A_{260} and A_{280} readings, but should not affect the performance of the DNA in downstream applications. Measure the absorbance at 320 nm, 280 nm, and 260 nm.

Subtract the absorbance reading obtained at 320 nm from the readings obtained at 260 nm and 280 nm to correct for the presence of magnetic particles.

- Concentration of DNA sample = 50 µg/ml x ($A_{260} – A_{320}$) x dilution factor
- Total amount of DNA purified = concentration x volume of sample in milliliters
- Purity of DNA sample = ($A_{260} – A_{320}$)/($A_{280} – A_{320}$).

* 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.

Ordering Information

Product	Contents	Cat. no.
BioSprint 15 DNA Plant Kit (360)	For 360 preps: 5-Rod Covers, 5-Tube Strips, MagAttract Suspension G, Buffers and Reagents	941517
BioSprint 96 DNA Plant Kit (576)	For 576 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	941557
BioSprint 96 Plant DNA Kit (1536)	For 1536 preps: Large 96-Rod Covers, 96-Well Microplates MP, S-Blocks, MagAttract Suspension G, Buffers and Reagents	941558
Buffer AE (240 ml)	240 ml Elution Buffer	19077
TissueLyser II and accessories		
TissueLyser II	Bead mill, 100-120/220-240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately)	85300
TissueLyser Adapter Set 2 x 24	2 sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96	2 sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
Tungsten Carbide Beads, 3 mm (200)	200 tungsten carbide beads (3 mm diameter), suitable for use with TissueLyser systems	69997
Stainless Steel Beads, 5 mm (200)	200 stainless steel beads (5 mm diameter), suitable for use with TissueLyser systems	69989

Product	Contents	Cat. no.
TissueLyser 3 mm Bead Dispenser, 96-well	For dispensing 96 beads (3 mm diameter) in parallel	69973
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
TissueLyser 5 mm Bead Dispenser, 96-well	For dispensing 96 beads (5 mm diameter) in parallel	69975
Collection Microtubes (racked, 10 x 96)	Nonsterile polypropylene tubes; 960 in racks of 96	19560
Collection Microtube Caps (120 x 8)	Nonsterile polypropylene caps for collection microtubes (1.2 ml) and round-well blocks; 960 in strips of 8	19566
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
96-Well Magnet Type A	Magnet for separating magnetic particles in wells of 96-well plates, 2 x 96-Well Microplates FB	36915
96-Well Microplates FB (24)	96-well microplates with flat-bottom wells for use with the 96-Well Magnet Type A; pack of 24	36985
Plate Rotor 2 x 96	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges	81031
Centrifuge 4-16S (120 V, 60 Hz)	Universal laboratory centrifuge with brushless motor (120 V, 60 Hz)	81510*
Centrifuge 4-16KS (220–240 V)	Refrigerated universal laboratory centrifuge with brushless motor (220–240 V, 50/60 Hz)	81610*

* Visit **www.qiagen.com** for more information about centrifuges with different V/Hz ratings, suitable for your region.

Product	Contents	Cat. no.
DNeasy® Plant Kits – for purification of total cellular DNA from plant cells and tissues or fungi		
DNeasy Plant Mini Kit (50)	50 DNeasy Mini Spin Columns, 50 QIAshredder Mini Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69104
DNeasy Plant Mini Kit (250)	250 DNeasy Mini Spin Columns, 250 QIAshredder Mini Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69106
DNeasy Plant Maxi Kit (24)*	24 DNeasy Maxi Spin Columns, 24 QIAshredder Maxi Spin Columns, RNase A, Buffers, Collection Tubes (50 ml)	68163
DNeasy 96 Plant Kit (6)†	For 6 x 96 DNA minipreps: 6 DNeasy 96 Plates, Buffers, Reagents, RNase A, S-Blocks, Collection Microtubes (1.2 ml), Caps, AirPore Tape Sheets	69181
RNeasy® Plant Kits – for purification of up to 100 µg total RNA from plants and fungi		
RNeasy Plant Mini Kit (20)	20 RNeasy Mini Spin Columns, 20 QIAshredder Mini Spin Columns, Collection Tubes (1.5 and 2 ml), RNase-free Reagents and Buffers	74903
RNeasy Plant Mini Kit (50)	50 RNeasy Mini Spin Columns, 50 QIAshredder Mini Spin Columns, Collection Tubes (1.5 and 2 ml), RNase-free Reagents and Buffers	74904

* Requires use of a centrifuge capable of attaining 4500 x *g* equipped with a swing-out rotor for 50 ml centrifuge tubes.
† Requires use of the QIAGEN 96-Well-Plate Centrifugation system.

Product	Contents	Cat. no.
MagAttract 96 DNA Plant Core Kit – for efficient, high-throughput purification of total cellular DNA from plant tissue		
MagAttract 96 DNA Plant Core Kit (6)*	MagAttract Suspension A and buffers for 6 x 96 minipreps	67161

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* Requires use of the QIAGEN 96-Well-Plate Centrifugation system and a mixer mill, such as the TissueLyser II. Manual procedures require use of a 96-well compatible magnet.

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Notes

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