EchoLUTION™ Plant DNA Kit

96-well plate kits

USER MANUAL

RUO

REF

010-103-002

010-103-008

BioEcho Life Sciences GmbH | BioCampus Cologne, Main Building | Nattermannallee 1 | 50829 Cologne, Germany

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1. INTENDED PURPOSE

The BioEcho EchoLUTION Plant DNA Kit is intended for easy and efficient DNA extraction from plant tissue. The excellent yield and purity of total DNA obtained with the EchoLUTION Plant DNA Kit allows use in downstream applications without further processing.

The EchoLUTION Plant DNA Kit is intended for research use only.

2. EXPLANATION OF THE KIT

The EchoLUTION Plant DNA Kit is characterized by the EchoLUTION single-step purification technology and tailored lysis. Together they reduce the overall extraction time and workflow to a minimum with consistent results and sensitivity compared to state-of-the-art methods.

The EchoLUTION Plant DNA Kit benefits are:

- Short processing time
- Few protocol steps
- High sample throughput with minor equipment and capital investment
- Up to 56 % less plastic waste compared to conventional methods
- No toxic reagents

For further details about kit specifications, see Table 1.

Table 1: ***Kit specifications***

Specification	Description
Sample input	2–30 mg of plant material depending on sample type
Sample type	Plant leaves or seeds
Sample condition	Fresh, frozen, dried, or freeze-dried
Purified nucleic acid	DNA
Elution volume	100 µL
Expected yields	Up to 10 µg depending on plant species

2.1. *Single-step purification principle*

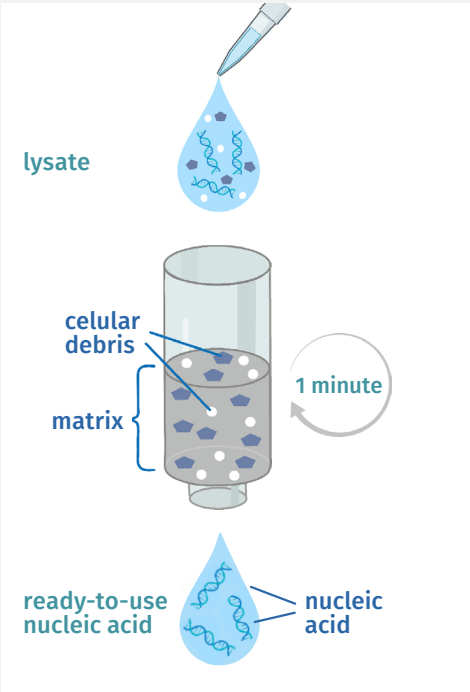
All BioEcho nucleic acid extraction kits are based on the EchoLUTION technology, which consists of tailored sample processing that includes lysis followed by single-step purification. The purification step works differently than conventional methods such as magnetic-bead and silica kits based on the bind–wash–elute method, and therefore needs only one centrifugation step.

The lysate is transferred onto the Spin Column or Purification Plate.

In a one-minute centrifugation step, nucleic acids pass through the purification matrix without interaction.

Impurities are held back and thereby completely removed.

The nucleic acids are in the flow-through and ready-to-use.



2.2. General comments

Comparison of the EchoLUTION technology to silica technologies– general aspects and handling

Using the EchoLUTION technology, nucleic acids are not bound to a membrane or magnetic beads and can migrate freely through the filter matrix. Unwanted components of the lysate are removed from the sample by remaining in the purification matrix.

The advantages of the EchoLUTION technology are:

1. No time-consuming washing steps
2. Easy handling
3. Reduced plastic waste

In contrast, silica technologies are based on the principle of adsorption. Here, the nucleic acids present in the lysate bind to a silica surface (membrane, magnetic beads), while unwanted cell components are removed by repeated washing with chaotropic and alcohol-containing wash buffers. Eventually, the nucleic acids are eluted with an aqueous buffer. Due to the repeated washing steps, silica-based methods are time-consuming, labor-intensive, and environmentally unfriendly.

Handling of purification matrix

The EchoLUTION purification matrix within the Purification Plate must be kept undamaged after the conditioning step to avoid short-circuit currents. Short-circuit currents result in the introduction of lysis components into the eluate and inadequate purification, which can lead to inhibition in downstream analysis. Therefore, when applying the lysate to the column **avoid touching the surface** of the filter matrix after conditioning and pipet **the sample very slowly (ideally dropwise)**.

To guarantee proper handling of the Purification Plate, be sure to use the recommended *g*-force centrifuge settings. Most centrifuges offer the choice between rpm and *g*-force (rcf); if rcf is not available, calculate the rpm corresponding to the required *g*-force using the calculator in the link or QR code below:

http://www.geneinfinity.org/sp/sp_rotor.html

For support on suitable centrifuges, please contact us.

Handling DNA

In general, good laboratory practices should always be used when working with DNA. The most common sources of contamination are dust and hands, as they can hold e.g., bacteria and molds. Therefore, always wear gloves while handling reagents and samples. Replace gloves regularly and keep tubes closed when possible.

Bench surfaces, laboratory equipment (e.g., pipettes and electrophoresis tanks), and non-disposable plasticware can be decontaminated using general laboratory reagents. Plasticware can be decontaminated with commercially available DNase removing solutions.

It is recommended to store purified DNA in a tightly capped tube at 2–8 °C for 24 hours or at –20 °C for long-term storage.

Homogenization of plant material

Plants present an additional layer of complexity when extracting DNA. Their cells contain not only a cell membrane but also a cell wall. Therefore, an efficient mechanical disruption and homogenization of the plant material is essential to ensure a high DNA yield during extraction.

The EchoLUTION Plant DNA Kit, optimized for plant leaves and seeds, is compatible with other plant tissues such as roots. Several methods will allow you to achieve efficient homogenization and tissue disruption. You can grind your samples with a pestle and mortar using liquid nitrogen. However, if you want to prepare numerous plant samples, we recommend using a homogenizer and processing them using a stainless-steel bead and liquid nitrogen. Additionally, establishing empirical homogenization might be needed for unique plant species depending on tissue type, sample amount, bead size, and equipment.

Homogenizer methods:

- Mixer Mills (Retsch): Place plant material (dried or frozen) with a stainless-steel bead (4 mm) and ensure the sample is closed. Depending on plant material, homogenize samples from 60–180 seconds at 30 Hz (1,800 rpm) or until forming plant powder. Homogenize fresh sample material with the Bead Beating Buffer Plant as described in the protocol.
- FastPrep® Instruments (MP Biomedicals®) or Precellys® Evolution Touch (Bertin Technologies): Homogenization time and speed might vary depending on the plant species and instrument. You can establish the optimal conditions depending on your plant sample or try the predefined protocols. Please refer to the equipment manufacturer's recommendations for further protocol setups and instructions.

High throughput automation

The EchoLUTION Plant DNA Kit has been demonstrated on the Hamilton Microlab STAR liquid handling platform to purify DNA for 96-well formats in a fully or semi-automated system. Other liquid handling platforms can be used for DNA extraction. We will be happy to work together to develop tailored automated protocols that fit your needs. Please, contact us to get more information about automated solutions.

3. MATERIALS

3.1. Materials provided

Table 2: ***Content of EchoLUTION Plant DNA Kit, 96-well plate format***

Product number	010-103-002	010-103-008
Product name	**EchoLUTION Plant DNA Kit (2 × 96)**	**EchoLUTION Plant DNA Kit (8 × 96)**
Reactions	192	768
TurboLyse Protease Plant (P)	1 × 1 mL	2 × 2 mL
RNase Plant (R)	1 × 200 µL	1 × 800 µL
Bead Beating Buffer Plant (BB)	1 × 20 mL	1 × 80 mL
Lysis Buffer Plant (LB)	1 × 20 mL	1 × 80 mL
Clearing Solution Plant (CS)	1 × 5 mL	1 × 20 mL
Low-TE Buffer (T)	1 × 1.2 mL	2 × 1.2 mL
Purification Plate 96 Type 1	2 plates	8 plates
Elution Plate 96 Type 1	2 plates	8 plates
Sealing Foil	2 foils	8 foils
Adhesive Foil	2 foils	8 foils

3.2 Materials required but not provided

A. Conditioning Plate

The Conditioning Plate is necessary to remove the matrix storage buffer from the Purification Plate. The Conditioning Plate can be reused up to 20 times. Please remove the collected buffer after every use. Please do not use any alternative plate. The plate needs to be ordered separately. To purchase this item, use the product number 060-001-002 or 060-001-008, depending on the number of plates required.

B. BioEcho Steel Beads

The stainless-steel beads of 4 mm are used for fast and efficient plant tissue disruption and sample homogenization. To purchase this item, use the product numbers 050-006-002 and 050-006-010, depending on the number of beads required to process your samples.

C. Tubes for master mixes

These tubes are required for the preparation of master mixes.

D. Multichannel reagent reservoir

These reservoirs are necessary when using multichannel pipettes for transferring prepared master mixes or buffers.

E. Lysis plate

The lysis plate necessary for sample homogenization and lysis is not included in the kit. We offer two suitable accessories for sale. Depending on your thermal shaker system, you can use the Tube & Cap Strips (product number: 060-002-008) or the Lysis Plate 96, Type 1 (product number: 060-003-008). The Lysis Plate 96, Type 1 is compatible with the Eppendorf® ThermoMixer® C using the SmartBlock DWP 1000 adaptor. However, you can also use a lysis plate of preference suitable for your system. In this case, the lysis plate should be a 96-well plate with a capacity of at least 1 mL per well, and it must be able to close each well independently to avoid cross-contamination during sample homogenization and lysis.

F. Plates for counterbalance in centrifuge

In case an odd number of plates is processed, prepare an additional plate stack to ensure the centrifuge is balanced and fill the wells with the appropriate amount of water.

3.3 Laboratory equipment needed

A. Plate centrifuge

For the procedure, plate centrifuges with the following specifications are mandatory:

- Standardized Society for Biomolecular Screening (SBS) format
- Capable of at least 4,500 x *g*.
- Capable of holding plate stacks of 5 cm height
- Swing-out rotor

B. Pipetting equipment

Pipetting can be performed using a single-channel pipette as well as a multi-channel pipette for pipette for 20 µL up to 100 or 200 µL up to 1,000 µL. We recommend using wide-bore tips for mixing and transferring the lysate to the purification matrix.

C. Thermal shaker for plates

The thermal shaker is used for the lysis step. It needs to reach up to 80 °C and form 1,200–2,000 rpm (e.g., Eppendorf ThermoMixer C, using the SmartBlock DWP 1000 adaptor). Alternatively, you can use a heating block or heat chamber.

D. Homogenizer

Required for plant tissue disruption and sample homogenization. Suitable for single tubes, racks or plate formats (see section 2.2.)

4. STORAGE AND STABILITY

4.1. Kit and reagents

- The EchoLUTION Plant DNA Kit is shipped at ambient temperature.
- Upon kit arrival, the Purification Plates and the Enzyme Box should be stored at 2–8 °C. The other kit components are stable at room temperature (15–25 °C).
- The Purification Plates need to be stored as described on the label (label facing up).

Table 3: ***Stability of EchoLUTION Plant DNA Kit components***

Component	Stability
TurboLyse Protease Plant **RNase Plant**	Stable at 2–8 °C until expiration date mentioned on label
Bead Beating Buffer Plant **Lysis Buffer Plant** **Clearing Solution Plant** **Low-TE Buffer**	Stable at 2–25 °C until expiration date mentioned on label
Purification Plates	Unopened stable at 2–8 °C until expiration date mentioned on label
Elution Plates **Adhesive foils** **Sealing foils**	Stable at 2–25 °C until expiration date mentioned on label

4.2. Sample collection

Plant samples can be collected and processed fresh, frozen, dried, or freeze-dried. Please make sure to use the correct protocol depending on your sample and storage method.

4.3. Storage and stability of purified nucleic acids

For long-term storage of purified nucleic acids, it is recommended to store the DNA samples at –20 °C.

5. WARNINGS AND SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please refer to the instructions supplied with our safety data sheets (SDS). Please contact us for the SDS.

Table 4: ***EchoLUTION Plant DNA Kit safety information***

Component	Hazardous component	GHS symbol	Hazard statements	Precautionary statements	Additional statement
TurboLyse Protease Plant (P)	Subtilisin	 Danger	H315; H318; H334; H335, H400, H411	P101; P102; P103; P284; P303+P361+P353; P305+P351+P338; P310; P405; P501	-
Clearing Solution (CS)	Strontium chloride	 Danger	H318	P101; P102; P103; P280; P305+P351+P338; P310; P501	-

Hazard Statements

H315: Causes skin irritation.
H318: Causes serious eye damage.
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335: May cause respiratory irritation.
H400: Very toxic to aquatic life.
H411: Toxic to aquatic life with long lasting effects.

Precautionary statements

P101: If medical advice is needed, have product container or label at hand.
P102: Keep out of reach of children.
P103: Read carefully and follow all instructions.
P280: Wear eye protection / face protection.
P284: In case of inadequate ventilation] wear respiratory protection.
P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310: Immediately call a POISON CENTER/doctor.
P405: Store locked up.
P501: Dispose of contents/container in accordance with local/regional/national/international regulations.

6. DISPOSAL

Please follow local regulations regarding the collection and disposal of hazardous waste and contact your waste disposal company to obtain information on laboratory waste disposal (waste code number 16 05 06). For further information, please refer to the instructions supplied with our SDS. Please contact us for the SDS.

Dispose of biological samples as biohazardous waste and all remaining liquid waste generated during the purification procedure according to local regulations.

A. Components and Purification Plates

Components that have come into contact with potentially infectious material should be autoclaved. Used components may retain some buffer residues, which should be disposed according to local/regional/national/international regulations.

The storage buffer of the Purification Plates collected in the Conditioning Plate during the conditioning step can be disposed of in the sink or on a paper tissue.

7. PROTOCOL

This protocol has been developed to extract DNA from plant tissues using the EchoLUTION Plant DNA Kit and is suitable for fresh and frozen plant tissues like leaves and seed samples. The input material amount might vary from 10–30 mg depending on tissue type and plant species. Therefore, an assessment of the ideal amount of input material may be required.

This is not a silica-based kit. Please read the instructions carefully before starting!

Preparation before starting:

- Pre-heat the thermal shaker to 60 °C.
- Set centrifuge to 1,000 x *g*.
- Carry out the complete DNA extraction at room temperature.

IMPORTANT NOTES:

- *Choose x g (rfc), not rpm, unless stated otherwise.*

1. Sample disruption and homogenization

a) Fresh sample material

- Transfer up to 30 mg plant material and a 4 mm steel bead (not provided, product numbers 050-006-002 or 050-006-010) into each well of the lysis plate (not provided, product numbers: 060-003-008 or 060-002-008).
- Add 100 µL Bead Beating Buffer Plant (BB) to each sample and close the lysis plate. If you are using the Tube & Cap Strips or the Lysis Plate 96, Type 1 from BioEcho, close with the Cap Strips.
- Place the lysis plate in the homogenizer for sample disruption. See section 2.2. for further information on homogenization. You might have to optimize the homogenization depending on plant species, tissue type, sample amount, bead size, and equipment.

- Centrifuge lysis plate for 1 minute at 1,000 x *g*, to collect the sample material at the bottom of the well and reduce foam formation.
- Prepare the lysis mix (according to table 5) with additional reactions to avoid buffer shortage.

Table 5: ***Lysis mix for fresh sample material***

Component	Volume
Lysis Buffer Plant (LB)	95 µL
TurboLyse Protease Plant (P)	5 µL
Final volume (µL)	100 µL

- Add 100 µL of the lysis mix to each well. Resuspend the sample by pipetting up and down. Seal the lysis plate tightly with the Adhesive Foil and proceed to step 2.

b) Frozen sample material and seeds

- Transfer up to 30 mg plant material and a 4 mm steel bead (not provided, product numbers 050-006-002 or 050-006-010) into each well of the lysis plate (not provided, product numbers: 060-003-008 or 060-002-008) and close. If you are using the Tube & Cap Strips or the Lysis Plate 96, Type 1 from BioEcho, close with the Cap Strips.
- Place the Lysis Plate in the homogenizer for sample disruption. See section 2.2. for further information on homogenization. You might have to optimize the homogenization depending on plant species, tissue type, sample amount, bead size, and equipment.

- Centrifuge the lysis plate for 1 minute at 1,000 x *g*, to collect the sample material at the bottom of the well.
- Prepare the lysis mix (according to table 6) with additional reactions to avoid buffer shortage.

Table 6: ***Lysis mix for frozen sample material and seeds***

Component	Volume
Lysis Buffer Plant (LB)	95 µL
TurboLyse Protease Plant (P)	5 µL
Final volume (µL)	100 µL

- Add 100 µL of the lysis mix to each well. Resuspend the sample by pipetting up and down. Seal the lysis plate tightly with the Adhesive Foil and proceed to step 2.

2. Sample lysis

- Place the Lysis Plate in the thermal shaker and incubate for 30 minutes at 60 °C with constant shaking of minimum 1,200 rpm to maximum 2,000 rpm. If agitation is not feasible, prolong incubation up to 60 minutes and mix samples by vortexing them from time to time.

NOTE:

- *Mixing has a major impact on the lysis efficiency. Therefore, if agitation is not feasible, prolong the incubation time as recommended and vortex samples from time to time. Please contact us if you need support to tailor the lysis to your equipment.*

- After incubation at 60 °C, increase the temperature to 80 °C and incubate for an additional 10 minutes with constant shaking of minimum 1,200 rpm to maximum 2,000 rpm.

NOTE:

- *During incubation, proceed with step 3. Purification Plate preparation. Additionally, prepare the clearing mix (according to table 7).*

Table 7: ***Clearing mix***

Component	Volume
RNase Plant (R)	1 µL
Clearing Solution Plant (CS)	24 µL
Final volume (µL)	25 µL

3. Purification Plate preparation

- Detach first the lower and then the upper foil from the Purification Plate. Be sure to keep the plates in a horizontal position while removing the foils, as the wells contain liquid.
- Place the Purification Plate on top of the Conditioning Plate (not provided, product number 060-001-002 or 060-001-008).

- Centrifuge plate stack for 1 minute at 1,000 x *g*, discard flow-through.
- Place the Purification Plate on top of the Elution Plate.
- Proceed directly with step 4.

NOTES:

- *The centrifuge rotor should be capable of holding plate stacks that have a height of 5 cm.*
- *Conditioning Plates can be reused.*
- *If the Purification Plate was not shipped or stored upright, the matrix may stick to the upper foil. In this case, shake plate until the matrix is removed from upper foil.*
- *Make sure the foil is completely removed from the bottom.*

4. DNA purification

- Allow samples to cool down at room temperature for minimum 5 minutes. If you want to speed up the process, you can place the samples on ice for 1 minute.
- Add 25 µL of clearing mix to each well. The sample will become cloudy. Vortex shortly or mix samples thoroughly and incubate them for 2 minutes at room temperature.

- Centrifuge lysis plate for 3 minutes at maximum speed (up to 5,000 x *g*) and transfer up to 100 µL of the clear lysate to the Purification Plate.

IMPORTANT NOTE:

- *The use of wide-bore tips is recommended for mixing and transfer of the lysate to the Purification Plate.*
- *Pipet slowly, drop-by-drop, and vertically onto the middle of the wells to not destroy the matrix surface (use an 8-channel pipette or robot).*
- *Do not touch the matrix bed with the pipette tip during sample loading!*

- Centrifuge the plate stack (Purification Plate on top of the Elution Plate) for 1 minute at 1,000 x *g*.
- Purified DNA is in the flow-through and ready-to-use.

NOTE:

- *The supplied Adhesive Foil cannot be used for the storage of nucleic acids.*

The extracted DNA can be used immediately or stored. For long-term storage, seal Elution Plate with Sealing Foil and place your DNA samples at –20 °C.

IMPORTANT NOTE:

- *For spectrophotometric analysis, use the Low-TE Buffer supplied with the kit as blank.*

7.1. Alternative protocol for dried and freeze-dried sample material

To process dried or freeze-dried plant samples, use 2–10 mg of input material and follow the protocol for sample disruption and homogenization for frozen material and seeds (step 1. b). Then proceed with step 2. The input material amount might vary depending on tissue type and plant species. Therefore, an initial assessment of the ideal amount of input material may be required depending on your plant species.

7.2. Quick protocol EchoLUTION™ Plant DNA Kit: 96-well plate kits

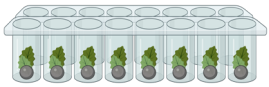
IMPORTANT NOTE: *Please use the quick protocol only after you have read and understood the complete user manual.*

Preparation before starting:

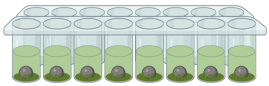
- Pre-heat the thermal shaker to 60 °C.
- Set the microcentrifuge to 1,000 x *g*.

1. Sample disruption and homogenization

a) Fresh sample material

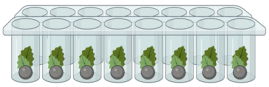


- Transfer up to 30 mg of plant material and a steel bead into each well of the lysis plate.
- Add 100 µL of the BB and close lysis plate.
- Homogenize samples according to the instrument manufacturer's recommendations.
- Centrifuge lysis plate for 1 min at 1,000 x *g*.

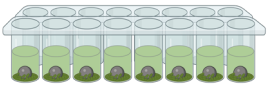


- Add 100 µL of the lysis mix:
  - LB: 95 µL
  - P: 5 µL
- Seal lysis plate with Adhesive Foil.

b) Frozen sample material

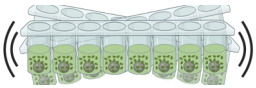


- Transfer up to 30 mg of plant material and a steel bead into each well of the lysis plate.
- Close lysis plate and homogenize samples according to the instrument manufacturer's recommendations.
- Centrifuge lysis plate for 1 min at 1,000 x *g*.



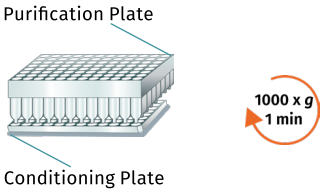
- Add 100 µL of the lysis mix:
  - LB: 95 µL
  - P: 5 µL
- Seal lysis plate with Adhesive Foil.

2. Sample lysis



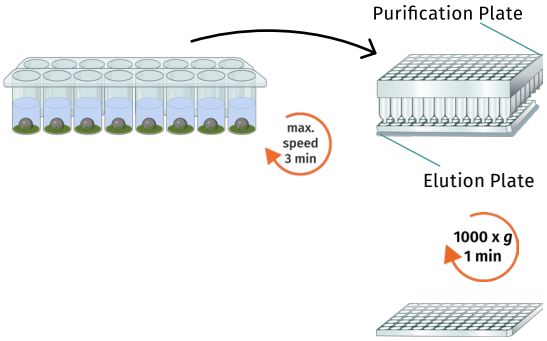
- Incubate at 60 °C for 30 min with constant shaking of 1,200–2,000 rpm.
- Incubate at 80 °C for 10 min with constant shaking of 1,200–2,000 rpm.
- Prepare clearing mix and proceed to step 3.
  - R: 1 µL
  - CS: 24 µL

3. Purification Plate preparation



- Detach first the lower and then the upper foil from the Purification Plate.
- Place the Purification Plate on top of the Conditioning Plate.
- Centrifuge plate stack for 1 min at 1,000 x *g*. Discard the flow-through.
- Place the Purification Plate on top of the Elution Plate.

4. DNA purification



- Cool down samples at RT for ~ 5 min, or place them on ice for 1 min.
- Add 25 µL of clearing mix, vortex shortly and incubate them for 2 min at RT.
- Centrifuge lysis plate for 3 min at full speed.
- Transfer 100 µL lysate. Pipet slowly, drop-by-drop onto the middle of the column without touching the matrix.
- Centrifuge 1 min at 1,000 x *g*.
- Purified DNA is in the flow-through and ready-to-use.

8. QUALITY CONTROL

Following the BioEcho Quality Management System, each lot of the EchoLUTION Plant DNA Kit is tested against predetermined specifications to ensure consistent product quality.

To request the Certificate of Analysis (CoA), please contact us.

9. TROUBLESHOOTING

Observation	Comments and suggestions
DNA yield and concentration is low	**Sample condition** Plant age and condition might influence the amount of extracted DNA. Therefore, we recommend verifying that the plant material is fresh and does not present any visible damage. Additionally, long-term storage of fresh samples can contain high amounts of degraded DNA. **Sample input** Use the correct amount of plant input to ensure appropriate experimental conditions. Depending on the plant species, the optimal amount may vary. If the plant amount described in the protocol is not working or you want to extract DNA from a plant species you have not worked with, you might need to run an assessment to determine the optimal input quantity required for the extraction. For questions about complicated-to-extract or new plant species samples, please contact us. **Incomplete sample disruption** Insufficient sample disruption and homogenization can lead to inefficient sample lysis and low DNA content. Please make sure to disrupt your sample completely, following the protocols described for your sample type (fresh or dried) and following the recommendations given in section 2.2.

DNA yield and concentration is low (continuation)

Incomplete sample lysis

The complete lysis of plant material highly depends on factors like sample input and homogenization, as described above. Moreover, it is necessary to guarantee a thorough mixing of samples during the enzymatic lysis. If reaching the mixing settings is unlikely, we recommend prolonging the sample lysis and mixing by vortexing during incubation.

Additionally, to guarantee that the TurboLyse Protease efficiency does not decrease, please ensure it is stored as described on the label. If used over a longer time, avoid exposing the enzyme to frequent temperature changes and store it in small aliquots.

When processing challenging samples or plant species, we suggest prolonging the lysis time or increasing the Lysis Buffer and enzyme concentration. Lysis Buffer and TurboLyse enzyme can be purchased separately (product number: 010-123-001 or 010-123-020).

Centrifuge settings

Most centrifuges offer the choice between rpm and *g*-force (rcf); if not available, calculate the rpm, see section 2.2. Always make sure to use the correct time mentioned in the protocol, to avoid insufficient passage through the matrix bed.

Adjust Purification Plate conditioning settings

For low-concentrated plant samples, we recommend increasing the centrifugation speed during conditioning to 2,000 x *g* for 1 minute.

Loading of purification matrix

The correct loading of the column is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the column to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading.

A_{260}/A_{280} and A_{260}/A_{230} values are low	**Wrong blank in measurements** Use supplied Low-TE Buffer (T) as blank in measurements. **Incorrect lysate volume** Avoid overloading the purification matrix by increasing lysate volume. Using a higher volume than the one recommended in the protocol will compromise the sample purity. **Low DNA concentrations** Spectrophotometrically measured 260/230 ratios are dependent on the DNA concentration. The reliability of the 260/230 ratios is compromised for low-concentrated samples (< 20 ng/µL) due to the low absorption peak at 260 nm that does not significantly reach above the background noise of the measurements. As a result, the overall ratio is lower than the expected 2–2.2., even though the DNA is perfectly suitable for any downstream applications. **Sample input** Use the correct amount of plant input to ensure appropriate experimental conditions. Depending on the plant species, the optimal input amount may vary. Sometimes, less is better, as an increased amount of plant material per reaction may increase the DNA concentration but will compromise the purity of the extracted DNA. Matrix overloading can lead to a light green eluate and carryover of secondary metabolites.
Eluted DNA has a light green color	**Sample input** Colored eluates may indicate overloading of the column. We strongly recommend using the correct amount of plant input to ensure appropriate experimental conditions. Depending on the plant species, the optimal input amount may vary. Sometimes, less is better, as an increased amount of plant material might overload the column. Alternatively, you could reduce the lysate input up to 70 µL.

Poor performance in downstream experiments

Loading of purification matrix

The correct loading of the column is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the column to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading. Disruption of the matrix bed might cause short-circuit currents, which result in the introduction of lysis components into the eluate and inadequate purification.

Sample input

Use the correct amount of plant input to ensure appropriate experimental conditions. Depending on the plant species, the optimal input amount may vary. Sometimes, less is better, as an increased amount of plant material per reaction may increase the DNA concentration but will compromise the purity of the extracted DNA. Matrix overloading can lead to a light green eluate and carryover of secondary metabolites. Therefore, if you see inhibition or dropouts in your downstream applications, we recommend reducing your sample input and or increasing the Lysis Buffer Plant volume.

Sample homogenization

Using prolonged and increased sample homogenization settings might lead to DNA fragmentation, causing a reduction in the total DNA concentration and yield. Moreover, processing samples rich in secondary metabolites or polysaccharides with a thorough homogenization might increase the number of inhibitors in the lysate, causing interferences with your downstream applications. Thus, we recommend starting with the recommended time and speed settings (section 2.2.) and modifying them depending on your plant species.

Centrifuge settings

Most centrifuges offer the choice between rpm and *g*-force (rcf); if not available, calculate the rpm see section 2.2. Always make sure to stick to the correct time mentioned in the protocol to avoid insufficient passage through the matrix bed.

Occurrence of cross-contamination	**Contaminated pipettes** The use of contaminated pipettes can lead to cross-contamination. BioEcho recommends a separate set of pipettes for sample preparation and PCR preparation, which should be cleaned thoroughly at regular intervals. **Centrifuge settings** Excessive deceleration of the centrifuge may lead to cross-contamination of the samples from one well into the other. Experience shows that this problem only occurs with deceleration times of 2–3 seconds. Ideally, the deceleration of the centrifuge should take between 15–20 seconds.
Eluate is missing or volume to low	**Inappropriate handling** Blocking pipette tips by plant debris during aspiration of the lysate supernatant may result in a lower transfer volume (< 100 µL) and, consequently, lower eluate volume. We recommend using wide-bore tips to transfer the 100 µL lysate for loading onto the column. **Centrifuge settings** Most centrifuges offer the choice between rpm and *g*-force (rcf); if not available, calculate the rpm see section 2.2. Always make sure to stick to the correct time mentioned in the protocol to avoid insufficient passage through the matrix bed.

For questions and further troubleshooting, please contact us!

10. LIMITATIONS OF USE

Limitations regarding EchoLUTION Plant DNA Kit are listed below:

- Strict compliance with the user manual is required for DNA purification. Following good laboratory practices is crucial for the successful use of the product. Appropriate handling of the reagents is essential to avoid contamination and impurities.
- The proof of principle for the EchoLUTION Plant DNA Kit was evaluated and confirmed using state-of-the-art qPCR and sequencing. Performance parameters are highly dependent on the quality of sample collection.
- The kit is for research use only.

11. SYMBOLS

The following table describes the symbols that appear on the labeling of the EchoLUTION Plant DNA products and in this user manual.

Table 8: ***EchoLUTION Plant DNA Kit symbols.***

Symbols	Description
	Manufacturer
	For research use only
	Product number
	Batch code
	Contains sufficient for < n > reactions
	Temperature limitation
	Do not reuse
	Expiration date
	Consult instructions for use

WE ARE INTERESTED IN YOUR EXPERIENCE WITH BIOECHO PRODUCTS!

With questions or suggestions or for further troubleshooting, please contact us.

Visit our website and shop for further information, tutorials, and application notes.

This user manual can be found in our shop on the corresponding product page.

Interested in publishing an application note with us? Please get in touch!

+49 221 99 88 97-0

contact@bioecho.de

BioEcho Life Sciences GmbH
BioCampus Cologne, Main Building
Nattermannallee 1
50829 Köln/Cologne, Germany

BioEcho Life Sciences, Inc.
400 Tradecenter Suite 6900
Woburn, MA 01801, USA

www.bioecho.com