EchoLUTION Plant DNA 96 Core Kit - Protocol

for 96-well plate purification of genomic DNA from **fresh** plant tissue samples

This protocol has been developed for **fresh** plant tissues like leaves, blossoms, fruits, roots, flour and seed samples.

For **freeze-dried and dried plant tissues**, please use the corresponding protocol also supplied with this kit.

Materials and equipment needed

Sample: 10-30 mg are recommended for fresh plant leaves etc. (depending on plant species).

Supplied with the kit:

- **Purification Plate:** 96-well plate containing the resin matrix for DNA purification
- **Elution Plate:** 96-well plate for the collection of the purified DNA
- **Adhesive Foils** for plate sealing during lysis
- All needed reagents and solutions

Not supplied with the kit:

- **Conditioning Plate**: 96-deep well plate with minimum of 800 µl well volume for the collection of void volume during preparation of **Purification Plate**. Reusable! BioEcho product no. 060-001-002
- **Tube Strips Lysis Plate:** 96-well plate for the lysis of the plant samples in a 96-well bead-beating device followed by incubation in a 96-well thermo shaker.
- **Cap Strips** for sealing of the **Tube Strips Lysis Plate** during bead-beating
- **96-well swing-out centrifuge** (preferentially with switch option to rcf (x g*))
  Important: Switch centrifuge to *relative centrifugal force, rcf* (x *g**); if this is not possible please use formula below* to calculate the conversion of round per minute (rpm) into rcf.
- **96-well plate thermal shaker** with **agitation** (for fastest performance), capable of heating to 60°C and 80°C. Alternatively: Heating Block or heat chamber
- **8-channel pipets** for 200 µl scale
- **Troughs** for Master Mix preparation holding > 10ml
- **Plate(s) to be used as tara** in the centrifuge in case an odd number of plates are processed
- **Bead-beater device** for 96-deep well plates
- **Steel beads**. BioEcho product no. 050-006-200
- **Ceramic blade scalpel** for cutting plant samples. BioEcho product no. 050-002-001

Preparation before starting

- Heat the thermal shaker, thermo block or heat chamber to 60°C.
- Set the microcentrifuge to **1,000 x g ***.
  Important: **Switch to relative centrifugal force, rcf (x g***, not rpm).

PROTOCOL:

Lysis

1. Transfer plant samples and steel beads in each cavity of the 96-well **Lysis Plate** (if using BioEcho`s steel beads, add one bead for each well).
2. Prepare the **Bead-beating Master Mix** for 96 bead-beating reactions with 20% excess volume in a reagent trough:

   Bead-beating Master Mix

No. of samples	1	96 (+20%)	Yours
BB Buffer BB	99	11,400	
R RNAse A Plant (µl)	1	115	
Final volume (µl)	**100**	**11,515**	

3. Add **100 µl of the Bead-beating Master Mix** from step 2 to each well of the **Lysis Plate** containing the plant samples and the steel beads with a 8-channel pipette.
4. Seal **Lysis Plate** with suitable **sealing option of choice**.
   Note: BioEcho recommends the **Tube Strips Lysis Plate & Cap Strips** (BioEcho product no. 060-002-024 for optimal bead-beating of fresh plant tissues and sealing of the lysis plate.
5. Place the sealed **Lysis Plate** in the bead-beater and beat **3 min at 30 Hz** or until the plant tissues are completely disrupted.
   Note: Depending on the rigidity of the specific tissue, beating time needs to be adjusted to receive a homogenous tissue paste.
6. Centrifuge Lysis Plate for **1 min at 1,000 x g** with the attached sealing to collect the lysate at the bottom of the well.
7. Prepare the **Lysis Master Mix** for 96 lysis reactions with 20% excess volume in reagent trough:

   Lysis Master Mix

No. of samples	1	96 (+20%)	Yours
LB 96 Plant Lysis Buffer	100	11,520	
P TurboLyse P Protease (µl)	5	580	
Final volume (µl)	**105**	**12,100**	

8. Add **105 µl** of the **Lysis Master Mix** from step 7 to each lysate well of the **Lysis Plate.**
   Note: If sample type is strongly absorbing liquid (e.g. seeds etc.), the amount of added Lysis Buffer needs to be increased to 200µl.
9. Seal **Lysis Plate** tightly with the **Adhesive Foil** (supplied with the kit).
10. Place the sealed **Lysis Plate** in the thermal shaker and incubate at **60°C for 30 min** with max. agitation speed (or for 60 min if agitation is not feasible).
    Meanwhile during lysis, proceed with step 12, "Preparation of Purification Plate"
11. After incubation at 60°C, increase temperature to **80°C** and incubate for additional **10 min** with max. agitation.

Preparation of Purification Plate (during steps 10 and 11)

12. Carefully detach the **lower** and **upper** sealing foils from the **Purification Plate**.
    Note: If the purification plate was not shipped or stored upright, resin may stick to the upper foil. In this case, shake plate until resin is removed from upper sealing foil.
13. Plate preparation: Place the **Purification Plate** on top of a 96-deep well plate ("Conditioning Plate", not supplied, minimum well volume of 800 µl) and centrifuge for **1 min** at **1,000 x *g**** to collect the void buffer from the purification resin in the **Purification Plate**. Discard the flow-through volume ("void volume") collected in the lower **Conditioning Plate** (Conditioning Plate can be re-used).
14. Place the conditioned **Purification Plate** on top of an **Elution Plate** for elution of the purified DNA. Continue with "Purification of DNA".

Purification of DNA

15. Detach **Adhesive Foil** from the incubated **Lysis Plate** and **add 25 µl Clearing Solution P** (CS) to each well of the **Lysis Plate** and mix by pipetting up & down.
    The sample will become cloudy.
16. Centrifuge **Lysis Plate** for **3 min** at **full speed** to sediment debris.
17. Transfer the **lysis supernatant (max. 100 µl)** onto the **Purification Plate**.
    Important loading instructions:
    - Do not touch the cellular debris at the bottom of the well while removing the supernatant to avoid clogging of the pipet tip (preferentially, wide bore tips). Residual tissue particles may be loaded and will not interfere with purification.
    - During the loading step, make sure that the 8-channel pipette releases the lysate solution **slowly and vertically**, non-angular onto the middle of the resin surface!
    - Do not punch pipette tip into the resin bed during loading of lysate!
18. After completion of the loading step, centrifuge **Purification Plate** on top of an **Elution Plate** as "plate sandwich".
19. Centrifuge for **1 min** at **1,000 x g***.
20. The purified DNA elutes into the **Elution Plate** and can be immediately applied in downstream applications.

The eluted DNA can be used immediately or stored at 4°C or for long-term storage at −20°C. For spectrophotometric analysis, use **Low TE Buffer** (T) supplied with the kit as blank.

Product use limitation

The EchoLUTION Plant DNA 96 Kit is for research use only. It is not registered or authorized to be used for diagnosis, prevention or treatment of a disease.

* Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm matching the g-force using the formula: rpm = 1,000 x $\sqrt{\left(\frac{g}{1.12\ x\ r}\right)}$, where r = radius of rotor in mm. and g the required g-force.
E. g., with a radius of 150 mm, the corresponding rpm to 1,000 x g is approx. 2,400 rpm.

EchoLUTION Plant DNA 96 Core Kit

for 96-well purification of genomic DNA from fresh plant tissue samples

Product no. (plates)	010-103-108 (8 x 96)
Kit contents	Purification Plate, Elution Plate, Adhesive Foil, Buffer BB, 96 Plant Lysis Buffer, TurboLyse P Protease, RNase A Plant, Clearing Solution P, Low TE Buffer
Related products	Conditioning Plate 060-001-002 | Steel beads 050-006-200 | Tube Strips Lysis Plate & Cap Strips 060-002-024 | Ceramic Blade Scalpels 050-002-001

Quick PROTOCOL (please read protocol first)

Bead-beating, Sample Lysis and Clearing

- Load 96well **Lysis Plate** with plant samples and steel beads
- Prepare **Bead-beating Master Mix** for 96 reactions + 20% vol. excess
- Add **100 µl Bead-beating Master Mix** to each well to the **Lysis Plate** and seal plate with **sealing option of choice**
- Bead-beat for **3 min** at **30 Hz**
- Centrifuge **Lysis Plate** for **1 min** at **1,000 x g***
- Prepare **Lysis Master Mix** for 96 rxns. + 20% vol. excess
- Add **105 µl Lysis Master Mix** to each lysis well of the **Lysis Plate**
- Seal **Lysis Plate** tightly with the **Adhesive Foil**
- Incubate **Lysis Plate** for **30 min at 60°C** with max. agitation
- Incubate **Lysis Plate** for **10 min at 80°C** with max. agitation
- Detach **Adhesive Foil** and add **25 µl** of (CS) per well of the **Lysis Plate** and mix by pipetting up & down
- Centrifuge **Lysis Plate** for **3 min** at **full speed**

Preparation of Purification Plate (during 60°C / 80°C incubation)

- Detach lower and upper foil from **Purification Plate**
- Place **Purification Plate** on top of a **Conditioning Plate** (deep well)
- Centrifuge **1 min** at **1,000 x g*** to elute void buffer from **Purification Plate**
- Place prepared **Purification Plate** on top of **Elution Plate**

Purification of DNA

- Transfer **lysis supernatant (max 100 µl)** to **Purification Plate**
- Centrifuge **1 min** at **1,000 x g*** to elute DNA into **Elution Plate**
- Eluted DNA is ready to use

BioEcho Life Sciences GmbH
Nattermannallee 1
50829 Köln (Cologne)/Germany

Phone: +49 (0) 221-99 88 97-0
E-Mail: contact@bioecho.de
www.bioecho.de

EchoLUTION Plant DNA 96 Core Kit - Protocol

for 96-well plate purification of genomic DNA from **dried** plant tissue samples

This protocol has been developed for **dried** and **freeze-dried** plant tissues like leaves, blossoms, fruits, roots, flour and seed samples.

For **fresh** plant tissues, please use the corresponding protocol also supplied with this kit.

Materials and equipment needed

Sample: 2 - 7 mg freeze-dried, dried leaf material recommended

Supplied with the kit:

- **Purification Plate:** 96-well plate containing the resin matrix for DNA purification
- **Elution Plate:** 96-well plate for the collection of the purified DNA
- **Adhesive Foils** for plate sealing during lysis
- All needed reagents and solutions

Not supplied with the kit:

- **Conditioning Plate**: 96-deep well plate with minimum of 800 µl well volume for the collection of void volume during preparation of **Purification Plate**. Reusable! BioEcho product no. 060-001-002
- **Lysis Plate:** 96-well plate for the lysis of the plant samples in a 96-well bead-beating device followed by incubation in a 96-well thermo shaker
- **96-well swing-out centrifuge** (preferentially with switch option to rcf (x g*))
  Important: Switch centrifuge to *relative centrifugal force, rcf* (x *g**); if this is not possible please use formula below* to calculate the conversion of round per minute (rpm) into rcf.
- **96-well plate thermal shaker** with **agitation** (for fastest performance), capable of heating to 60°C and 80°C. Alternatively: Heating Block or heat chamber
- **8-channel pipets** for 200 µl scale
- **Troughs** for Master Mix preparation
- **Plate(s) to be used as tara** in the centrifuge in case an odd number of plates are processed
- **Bead-beater device** for 96-deep well plates
- **Steel beads**. BioEcho product no. 050-006-200
- **Ceramic blade scalpel** for cutting plant samples. BioEcho product no. 050-002-001

Preparation before starting

- Heat the thermal shaker, thermo block or heat chamber to 60°C.
- Set the microcentrifuge to **1,000 x g ***.
  Important: **Switch to relative centrifugal force, rcf (x g***, not rpm).

PROTOCOL:

Lysis

1. Transfer plant samples and beads in each cavity of the 96-well **Lysis Plate** (if using BioEcho`s steel beads, add one bead for each well).
2. Seal **Lysis Plate** with suitable **sealing option of choice**.
   Note: BioEcho recommends the Lysis Plate Type 1 (BioEcho product no. 060-003-008) & Silicone mat (BioEcho product no. 050-009-008) for optimal bead-beating of dried plant tissues and sealing of the lysis plate.
3. Place the **Lysis Plate** in the bead-beater and beat **3 min at 30 Hz** or until the plant tissue is completely disrupted.
4. Centrifuge **Lysis Plate** for **1 min at 1,000 x g** with the sealing attached to collect the disrupted sample at the bottom of the well.
5. Prepare the **Lysis Master Mix** for 96 lysis reactions with 20% excess volume in a reagent trough:

Lysis Master Mix

No. of samples	1	96 (+20%)	Yours
BB Buffer BB (µL)	94	10,830	
R RNAse A Plant (µl)	1	115	
LB 96 Plant Lysis Buffer (µL)	100	11,520	
P TurboLyse P Protease (µl)	5	580	
Final volume (µl)	**200**	**23,045**	

6. Add **200 µl of the freshly prepared Lysis Master Mix** to each lysate well of the **Lysis Plate** containing the plant samples and resuspend the homogenized plant tissue in the Lysis Master Mix by vortexing.
7. Seal the **Lysis Plate** tightly with the **Adhesive Foil** (supplied with the kit).
8. Place the **Lysis Plate** in the thermal shaker and incubate at **60°C for 30 min** with max. agitation speed (or for 60 min if agitation is not feasible).
   Meanwhile during lysis, proceed with step 10, "Preparation of Purification Plate"
9. After incubation at 60°C, increase temperature to **80°C** and incubate for additional **10 min** with max. agitation.

Preparation of Purification Plate (during steps 8 and 9)

10. Carefully detach the **lower** and **upper** sealing foils from the **Purification Plate**.
    Note: If the purification plate was not shipped or stored upright, resin may stick to the upper foil. In this case, shake plate until resin is removed from upper sealing foil.
11. Plate preparation: Place the **Purification Plate** on top of a 96-deep well plate ("Conditioning Plate", not supplied, minimum well volume of 800 µl) and centrifuge for **1 min** at **1,000 x *g**** to collect the void buffer from the purification resin in the **Purification Plate**. Discard the flow-through volume ("void volume") collected in the lower **Conditioning Plate** (not supplied).
12. Place the conditioned **Purification Plate** on top of an **Elution Plate** for elution of the purified DNA. Continue with "Purification of DNA".

Purification of DNA

13. Detach **Adhesive Foil** from the incubated **Lysis Plate** and **add 25 µl Clearing Solution P** (CS) to each well of the **Lysis Plate** and mix by pipetting up & down.
    The sample will become cloudy.
14. Centrifuge the **Lysis Plate** for **3 min** at **full speed** to sediment debris.
15. Transfer the **lysis supernatant (max. 100 µl)** onto the **Purification Plate**.
    Important loading instructions:
    - Do not touch the cellular debris at the bottom of the well while removing the supernatant to avoid clogging of the pipet tip (preferentially, wide bore tips).
      Residual tissue particles may be loaded and will not interfere with purification.
    - During the loading step, make sure that the 8-channel pipette releases the lysate solution **slowly and vertically**, non-angular onto the middle of the resin surface!
    - Do not punch pipette tip into the resin bed during loading of lysate!
16. After completion of the loading step, centrifuge **Purification Plate** on top of an **Elution Plate** as "plate sandwich".
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Related products	Conditioning Plate 060-001-002 | Steel beads 050-006-200 | Lysis Plate Type 1 060-003-008 | Silicone mat 050-009-008 | Ceramic Blade Scalpels 050-002-001

Quick PROTOCOL (please read protocol first)

Bead-beating, Sample Lysis and Clearing

- Load 96well **Lysis Plate** with plant samples and steel beads
- Seal **Lysis Plate** with **sealing option of choice**
- Bead-beat for **3 min** at **30 Hz**
- Centrifuge **Lysis Plate** for **1 min** at **1,000 x g***
- Prepare **Lysis Master Mix** for 96 reactions + 20% vol. excess
- Remove sealing from **Lysis Plate**, add **205 µl Lysis Master Mix** to each lysis well to the **Lysis Plate** and resuspend homogenized plant tissue
- Seal **Lysis Plate** tightly with the **Adhesive Foil**
- Incubate **Lysis Plate** for **30 min at 60°C** with max. agitation
- Incubate **Lysis Plate** for **10 min at 80°C** with max. agitation
- Detach **Adhesive Foil** and add **25 µl** of (CS) per well of the **Lysis Plate** and mix by pipetting up & down
- Centrifuge **Lysis Plate** for **3 min** at **full speed**

Preparation of Purification Plate (during 60°C / 80°C incubation)

- Detach lower and upper foil from **Purification Plate**
- Place **Purification Plate** on top of a **Conditioning Plate** (deep well)
- Centrifuge **1 min** at **1,000 x g*** to elute void buffer from **Purification Plate**
- Place prepared **Purification Plate** on top of **Elution Plate**

Purification of DNA

- Transfer **lysis supernatant (max 100 µl)** to **Purification Plate**
- Centrifuge **1 min** at **1,000 x g*** to elute DNA into **Elution Plate**
- Eluted DNA is ready to use

BioEcho Life Sciences GmbH
Nattermannallee 1
50829 Köln (Cologne)/Germany

Phone: +49 (0) 221-99 88 97-0
E-Mail: contact@bioecho.de
www.bioecho.de