

LOT: See product label EXPIRY DATE: See product label

## ORDERING INFORMATION

PRODUCT	GenUP™ Plant DNA Kit		
CAT.NO.	BR0700801	BR0700802	BR0700803
SIZE	10 preps	50 preps	250 preps
COMPONENTS			
Buffer LYSIS LC	12 ml	25 ml	120 ml
Buffer LYSIS LS	10 ml	25 ml	125 ml
Buffer LYSIS LD	10 ml	25 ml	125 ml
Buffer BINDING BD	16 ml	16 ml	60 ml
Proteinase K (lyophilized)	1 vial (add 0.3 ml water)	1 vial (add 1.5 ml water)	4 vials (add 1.5 ml water)
Buffer PRECIPITATION	6ml	6ml	30 ml
Buffer WASH C (concentrate)	6 ml (add 14 ml ethanol)	24 ml (add 56 ml ethanol)	2 × 60 ml (add 140 ml ethanol)
Buffer ELUTION	2×2ml	15 ml	2×30 ml
Pre-Filters (lavender)	10	50	5×50
Mini Filters (green)	10	50	5×50
Collection Tubes (2 ml)	50	5×50	25×50
Elution Tubes (1.5 ml)	10	50	5×50

**STORAGE** 

Room temperature (until expiry date—see product label). If precipitation appears, gently warm the solution to dissolve the precipitate. Store lyophilized and dissolved Proteinase K at 2-8 °C.

## **FEATURES**

- Simple and efficient procedure for plant DNA isolation
- Special lysis protocols for different plant materials
- High yields of inhibitor-free DNA

## **APPLICATIONS**

• Universal kit for isolating genomic DNA from various plant materials

## **DESCRIPTION**

biotechrabbit™ GenUP Plant DNA Kit has been specially developed for quick and easy purification of genomic DNA from a wide variety of plant materials, including fresh, frozen or dried samples from leaves, roots, stems and flowers. The kit includes an advanced prefiltration step to remove unlysed tissue. Subsequently, DNA is bound to a Mini Filter and is subsequently washed and eluted in a separate tube. The purified DNA is ready for use in any demanding molecular biology application, including PCR, enzymatic digestions and cloning.

This kit provides three buffers for optimized processing with different plant materials. To determine optimal lysis conditions, side-by-side preparation using the three provided protocols are prepared.

## **SPECIFICATIONS**

STARTING MATERIAL	Fresh, frozen or dried plant tissue (maximum 100 mg dry weight or 180 mg wet weight)
EXTRACTION TIME	Approximately 40 min
BINDING CAPACITY	> 50 µg DNA
TYPICAL YIELD	Variable depending on the starting material; approximately 5–25 µg DNA
AVERAGE PURITY	A <sub>260</sub> /A <sub>280</sub> 1.7–2.0

## MATERIALS SUPPLIED BY THE USER

- 96-99.8 % ethanol
- Reaction tubes (1.5 ml or 2 ml)
- Pipet tips
- Double-distilled water
- Optional: RNase A (10 mg/ml)
   The Mini Filter binds both DNA and RNA. For isolation of RNA-free DNA, add RNase A after the lysis step.

## STEPS BEFORE STARTING

Add the following volume of 96–99.8% ethanol to each bottle Buffer WASH C, close firmly, mix thoroughly
and store at room temperature.

CAT. NO.	CONCENTRATE	ETHANOL	FINAL VOLUME
BR0700801	6 ml	14 ml	20 ml
BR0700802	24 ml	56 ml	80 ml
BR0700803	60 ml	140 m	200 ml

• Add the following volume of double-distilled water to each vial Proteinase K, mix thoroughly and store aliquots at 4 °C.

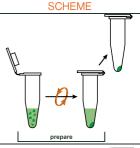
BR0700801	0.3 ml
BB0700802 BB0700803	15 ml etore as 5 x 0.3 ml aliquots

- Avoid freezing and thawing of starting material.
- Mark all vials and filters to avoid confusion when purifying multiple preps.
- Heat thermal mixer or water bath at 65 °C.
- Perform all centrifugation steps at room temperature.

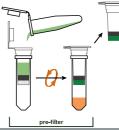
## SHORT PROTOCOL

STEPS

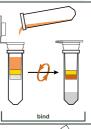
- Homogenize plant material.
  - o Mortar and pestle in presence of liquid nitrogen.
  - o Homogenizer, e.g., rotor-stator or bead mill.
- Lysis dependent on Buffer LYSIS:
  - o add Buffer LYSIS LC, incubate and proceed with the next step.
  - add Buffer LYSIS LS or Buffer LYSIS LD, incubate, add Buffer PRECIPITATION and centrifuge to remove unlysed material.



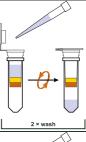
• Filter lysate using a Pre-Filter (black ring).



- Bind DNA to the Mini Filter (yellow ring) with Buffer BINDING BD.
- Centrifuge to remove unbound material.



- Wash the DNA twice with Buffer WASH C.
- Centrifuge twice to remove the wash buffer.



- Elute DNA with Buffer ELUTION and centrifuge.
- Purified DNA in the Elution Tube is ready for use.



## PROTOCOL FOR ISOLATION OF PLANT DNA USING BUFFER LYSIS LC

#### **PROCEDURE** NOTES Homogenize 50–100 mg plant material completely. Use 120–180 mg wet plant material. Transfer the homogenized material to a 1.5 ml or 2 ml For homogenization, use liquid nitrogen with a reaction tube. pestle and mortar or a commercial homogenizer. Add 400 µl Buffer LYSIS LC and 20 µl Proteinase K. • Before use, prepare Proteinase K as described above. Mix vigorously by pulse vortexing for 5 s. Incubate at 65 °C for 30 min. • Use a shaking platform (thermomixer, water bath or other rocking platform) to ensure continuous shaking during lysis. Alternatively, vortex the sample 3-4 times during the incubation. • When lysis is completed, the lysate becomes clear. • Transfer the cleared lysate to a Pre-Filter (black ring) • Do not discard the Collection Tube containing placed in a Collection Tube. the filtered lysate. Discard the reaction tube. Centrifuge at 10,000 × g (12,000 rpm) for 1 min to filter univsed material. Discard the Pre-Filter. • Optionally, add 4 µl RNase A (10 mg/ml, not included in the This step can be skipped if RNA-free DNA is kit), mix vigorously by pulse vortexing for 5 s, and incubate not required. 5 min at room temperature. Add 200 µl Buffer BINDING BD to the filtered lysate. For improved yield, mix thoroughly. Mix by vortexing or by pipetting up and down several times. • Transfer the sample to a Mini Filter (yellow ring) placed in • If the solution does not pass completely a new Collection Tube. through the Mini Filter, repeat the Centrifuge at 10.000 × q (12.000 rpm) for 2 min. centrifugation or use a longer centrifugation time. Discard the Collection Tube with the filtrate. Place the Mini Filter to a new Collection Tube. • Before use, prepare Buffer WASH Cas Add 650 ul Buffer WASH C to the Mini Filter. described above. Centrifuge at 10,000 × g (12,000 rpm) for 1 min. Discard the Collection Tube with the filtrate. Place the Mini Filter to a new Collection Tube. • Add 650 µl Buffer WASH C to the Mini Filter. Centrifuge at 10,000 × g (12,000 rpm) for 1 min. Discard the Collection Tube with the filtrate.

- Place the Mini Filter to a new Collection Tube.
- Centrifuge for 2 min at maximum speed to remove residual ethanol.
- Discard the Collection Tube.
- Place the Mini Filter into an Elution Tube.
- Add 100–200 µl Buffer ELUTION to the center of the Mini
- To improve yield, perform elution twice using ½ volume of Buffer ELUTION.

- Incubate at room temperature for 1 min.
- Centrifuge at 6,000 × g (8,000 rpm) for 1 min.
- Discard the Mini Filter.
- Purified DNA in the Elution Tube can be used immediately. Store the DNA at 4°C (short-term) or -20°C
  - (long-term).

## PROTOCOL FOR ISOLATION OF PLANT DNA USING BUFFER LYSIS LS

#### **PROCEDURE** NOTES • Homogenize 50–100 mg plant material completely. Use 120–180 mg wet plant material. Transfer the homogenized material to a 1.5 ml or 2 ml For homogenization, use liquid nitrogen with a reaction tube. pestle and mortar or a commercial homogenizer. Add 400 µl Buffer LYSIS LS. Use a shaking platform (thermomixer, water) bath or other rocking platform) to ensure Mix vigorously by pulse vortexing for 5 s. Incubate at 65 °C for 30 min. continuous shaking during lysis. Alternatively, vortex the sample 3-4 times during the incubation. • When lysis is completed, the lysate becomes clear. Add 100 µl Buffer PRECIPITATION and vortex for 5 s. Incubate at room temperature for 5 min. • Centrifuge at maximum speed for 5 min. Transfer the cleared lysate to a Pre-Filter (blackring) • Do not discard the Collection Tube containing placed in a Collection Tube. the filtered lysate. Discard the reaction tube. • If a pellet is present after centrifugation, Centrifuge at 10,000 $\times g$ (12,000 rpm) for 1 min to filter carefully transfer the supernatant to a new unlysed material. tube. Do not disturb the pellet. Discard the Pre-Filter. • Optionally, add 4 μl RNase A (10 mg/ml, not included in the This step can be skipped if RNA-free DNA is kit), mix vigorously by pulse vortexing for 5 s, and incubate not required. 5 min at room temperature. • Add 200 µl Buffer BINDING BD to the filtered lysate. • For improved yield, mix thoroughly. . Mix by vortexing or by pipetting up and down several times. Transfer the sample to a Mini Filter (vellow ring) placed in If the solution does not pass completely a new Collection Tube. through the Mini Filter, repeat the • Centrifuge at 10,000 × g (12,000 rpm) for 2 min. centrifugation or use a longer centrifugation time. Discard the Collection Tube with the filtrate. Place the Mini Filter to a new Collection Tube. • Before use, prepare Buffer WASH Cas described above. Add 650 µl Buffer WASH C to the Mini Filter. • Centrifuge at 10,000 × g (12,000 rpm) for 1 min. Discard the Collection Tube with the filtrate. Place the Mini Filter to a new Collection Tube. Add 650 ul Buffer WASH C to the Mini Filter. Centrifuge at 10.000 × q (12.000 rpm) for 1 min.

Discard the Collection Tube with the filtrate.

- Place the Mini Filter to a new Collection Tube.
- Centrifuge for 2 min at maximum speed to remove residual ethanol.
- Discard the Collection Tube.
- Place the Mini Filter into an Elution Tube.
- Add 100–200 µl Buffer ELUTION to the center of the Mini
- To improve yield, perform elution twice using ½ volume of Buffer ELUTION.

- Incubate at room temperature for 1 min.
- Centrifuge at 6,000 × g (8,000 rpm) for 1 min.
- Discard the Mini Filter.
- Purified DNA in the Elution Tube can be used immediately. Store the DNA at 4°C (short-term) or -20°C
  - (long-term).

## PROTOCOL FOR ISOLATION OF PLANT DNA USING BUFFER LYSIS LD

#### **PROCEDURE** NOTES Homogenize 50–100 mg plant material completely. Use 120–180 mg wetplant material. Transfer the homogenized material to a 1.5 ml or 2 ml For homogenization, use liquid nitrogen with a reaction tube. pestle and mortar or a commercial homogenizer. Add 400 µl Buffer LYSIS LD and 20 µl Proteinase K. • Before use, prepare Proteinase K as described above. Mix vigorously by pulse vortexing for 5 s. Incubate at 65 °C for 30 min. • Use a shaking platform (thermomixer, water bath or other rocking platform) to ensure continuous shaking during lysis. Alternatively, vortex the sample 3-4 times during the incubation. · When lysis is completed, the lysate becomes clear. Add 100 ul Buffer PRECIPITATION and vortex for 5 s. Incubate at room temperature for 5 min. Centrifuge at maximum speed for 5 min. Transfer the cleared lysate to a Pre-Filter (black ring) • Do not discard the Collection Tube containing placed in a Collection Tube. the filtered lysate. • Discard the reaction tube. • If a pellet is present after centrifugation, carefully transfer the supernatant to a new • Centrifuge at 10,000 × g (12,000 rpm) for 1 min to filter unlysed material. tube. Do not disturb the pellet. · Discard the Pre-Filter. • Optionally, add 4 µl RNase A (10 mg/ml, not included in the This step can be skipped if RNA-free DNA is kit), mix vigorously by pulse vortexing for 5 s, and incubate not required. 5 min at room temperature. Add 200 µl Buffer BINDING BD to the filtered lysate. • For improved yield, mix thoroughly. • Mix by vortexing or by pipetting up and down several times. • Transfer the sample to a Mini Filter (yellow ring) placed in • If the solution does not pass completely a new Collection Tube. through the Mini Filter, repeat the Centrifuge at 10.000 × q (12.000 rpm) for 2 min. centrifugation or use a longer centrifugation time. Discard the Collection Tube with the filtrate. Place the Mini Filter to a new Collection Tube. • Before use, prepare Buffer WASH Cas described above. Add 650 µl Buffer WASH C to the Mini Filter. • Centrifuge at 10,000 × g (12,000 rpm) for 1 min. Discard the Collection Tube with the filtrate.

- Place the Mini Filter to a new Collection Tube.
- Add 650 ul Buffer WASH C to the Mini Filter.
- Centrifuge at 10,000 × g (12,000 rpm) for 1 min.
- Discard the Collection Tube with the filtrate.

- Place the Mini Filter to a new Collection Tube.
- Centrifuge for 2 min at maximum speed to remove residual ethanol.
- Discard the Collection Tube.
- Place the Mini Filter into an Elution Tube.
- Add 100–200 µl Buffer ELUTION to the center of the Mini Filter.
- To improve yield, perform elution twice using ½ volume of Buffer ELUTION.

- Incubate at room temperature for 1 min.
- Centrifuge at 6,000 × g (8,000 rpm) for 1 min.
- Discard the Mini Filter.
- $\bullet \ \ \text{Purified DNA in the Elution Tube can be used immediately.} \quad \bullet \ \ \text{Store the DNA at 4°C (short-term) or -20°C}$ 
  - Store the DNA at 4°C (short-term) or -20°C (long-term).

	OOTING

PROBLEM	SOLUTION
CLOGGED MINI FILTER	
Too much starting material or insufficient lysis	Reduce the amount of starting material, and increase the lysis time. Increase the centrifugation speed. After lysis, centrifuge the lysate to pellet unlysed material.
LOWYIELD	
Insufficient lysis	Determine optimal lysis conditions using a side-by-side comparison using a single batch of homogenate.  Reduce the amount of starting material.  Do not overload the Mini Filter.
Incomplete elution	Increase the elution time up to 5 min, or repeat the elution. Use a higher elution volume or elute in two steps.
Insufficient mixing with Buffer BINDING BD	Mix the sample with Buffer BINDING BD by pipetting or vortexing before transferring to the Mini Filter.
LOW DNA CONCENTRATION	l .
Too much elution buffer used	Use less Buffer ELUTION.
SHEARED OR DEGRADED DY	NA
Incorrect storage of starting material	Freeze freshly collected samples in liquid nitrogen or at -20 °C to -80 °C. Store at -80 °C and avoid thawing before preparation.
Low-quality starting material	Avoid using old material.
RNACONTAMINATION	
No RNase treatment	The treatment with RNase is optional. If RNA-free material is required, perform RNase A digestion of the sample during the lysis or after elution.

## **SAFETY PRECAUTIONS**

- This kit is made for single use only!
- Don't eat or drink components of the kit!
- The kit shall only be handled by educated personal in a laboratory environment!
- Wear gloves while handling these reagents, and avoid skin contact! In case of contact, flush with water immediately!
- Handle and discard waste according to local safety regulations!
- Do not add bleach or acidic components to the waste after sample preparation!

## **CERTIFICATE OF ANALYSIS**

The components of the kit were tested for DNA purification from plant material, and the performance of purified DNA was confirmed in PCR amplification.

Quality confirmed by: Head of Quality Control

### SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS unter: http://www.biotechrabbit.com/support/documentation.html.

#### **USEFUL HINTS**

- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

#### CONTACT BIOTECHRABBIT

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