GenUP™ Plant DNA Kit

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	6 ml	6 ml	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 × 2 ml	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 Proteinase K at 4°C,
Store aliquots of dissolved Proteinase K at –20°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_{260}/A_{280} 1.7–2.0

MATERIALS SUPPLIED BY THE USER

- 96–99.8% ethanol
- Centrifugation tubes (1.5 ml)
- Pipet tips
- Double-distilled water
- *Optional:* RNase A (100 mg/ml)

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

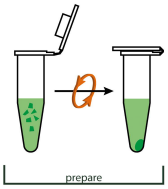
- Add the following volume of double-distilled water to each vial Proteinase K, mix thoroughly and store aliquots at –20°C.

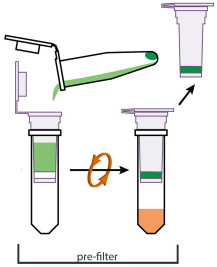
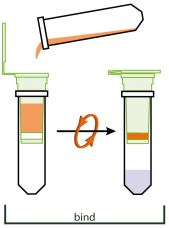
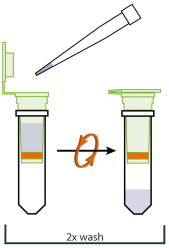
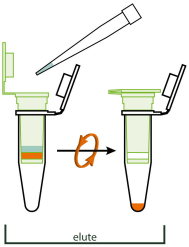
BR0700801	0.3 ml
BR0700802, BR0700803	1.5 ml for 5 × 0.3 ml aliquots

- 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	SCHEME

• Homogenize plant material.
• Mortar and pestle in presence of liquid nitrogen.
• Homogenizer, e.g., rotor-stator or bead mill.
• Lysis dependent on Buffer LYSIS:
  ○ 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 (lavender).	
• Bind DNA to the Mini Filter (green) 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. • Transfer the homogenized material to a 1.5 ml or 2 ml reaction tube.	• Use 120–180 mg wet plant material. • For homogenization, use liquid nitrogen with a pestle and mortar or a commercial homogenizer.
• Add 400 µl Buffer LYSIS LC and 20 µl Proteinase K. • Mix vigorously by pulse vortexing for 5 s. • Incubate at 65°C for 30 min.	• Before use, prepare Proteinase K as described above. • 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 (lavender) placed in a Collection Tube. • Discard the reaction tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min to filter unlysed material. • Discard the Pre Filter.	• Do not discard the Collection Tube containing the filtered lysate.
• *Optionally,* add 4 µl RNase A (100 mg/ml, not included in the kit), mix vigorously by pulse vortexing for 5 s, and incubate 5 min at room temperature.	• This step can be skipped if RNA-free DNA is not required.
• Add 200 µl Buffer BINDING BD to the filtered lysate. • Mix by vortexing or by pipetting up and down several times.	• For improved yield, mix thoroughly.
• Transfer the sample to a Mini Filter (green) placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Collection Tube with the filtrate.	• If the solution does not pass completely through the Mini Filter, repeat the centrifugation or use a longer centrifugation time.
• 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.	• Before use, prepare Buffer WASH C as described above.
• 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 Filter.
- Incubate at room temperature for 1 min.
- Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min.
- Discard the Mini Filter.

- To improve yield, perform elution twice using ½ volume of Buffer ELUTION.

- 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. • Transfer the homogenized material to a 1.5 ml or 2 ml reaction tube.	• Use 120–180 mg wet plant material. • For homogenization, use liquid nitrogen with a pestle and mortar or a commercial homogenizer.
• Add 400 µl Buffer LYSIS LS. • 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 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 (lavender) placed in a Collection Tube. • Discard the reaction tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min to filter unlysed material. • Discard the Pre Filter.	• Do not discard the Collection Tube containing the filtered lysate. • If a pellet is present after centrifugation, carefully transfer the supernatant to a new tube. Do not disturb the pellet.
• *Optionally,* add 4 µl RNase A (100 mg/ml, not included in the kit), mix vigorously by pulse vortexing for 5 s, and incubate 5 min at room temperature.	• This step can be skipped if RNA-free DNA is not required.
• Add 200 µl Buffer BINDING BD to the filtered lysate. • Mix by vortexing or by pipetting up and down several times.	• For improved yield, mix thoroughly.
• Transfer the sample to a Mini Filter (green) placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Collection Tube with the filtrate.	• If the solution does not pass completely through the Mini Filter, repeat the centrifugation or use a longer centrifugation time.
• 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.	• Before use, prepare Buffer WASH C as described above.
• 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 Filter.
- Incubate at room temperature for 1 min.
- Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min.
- Discard the Mini Filter.

- To improve yield, perform elution twice using ½ volume of Buffer ELUTION.

- 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. • Transfer the homogenized material to a 1.5 ml or 2 ml reaction tube.	• Use 120–180 mg wet plant material. • For homogenization, use liquid nitrogen with a pestle and mortar or a commercial homogenizer.
• Add 400 µl Buffer LYSIS LD and 20 µl Proteinase K. • Mix vigorously by pulse vortexing for 5 s. • Incubate at 65°C for 30 min.	• Before use, prepare Proteinase K as described above. • 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 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 (lavender) placed in a Collection Tube. • Discard the reaction tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min to filter unlysed material. • Discard the Pre Filter.	• Do not discard the Collection Tube containing the filtered lysate. • If a pellet is present after centrifugation, carefully transfer the supernatant to a new tube. Do not disturb the pellet.
• *Optionally,* add 4 µl RNase A (100 mg/ml, not included in the kit), mix vigorously by pulse vortexing for 5 s, and incubate 5 min at room temperature.	• This step can be skipped if RNA-free DNA is not required.
• Add 200 µl Buffer BINDING BD to the filtered lysate. • Mix by vortexing or by pipetting up and down several times.	• For improved yield, mix thoroughly.
• Transfer the sample to a Mini Filter (green) placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Collection Tube with the filtrate.	• If the solution does not pass completely through the Mini Filter, repeat the centrifugation or use a longer centrifugation time.
• 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.	• Before use, prepare Buffer WASH C as described above.
• 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 Filter.
- Incubate at room temperature for 1 min.
- Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min.
- Discard the Mini Filter.
- To improve yield, perform elution twice using ½ volume of Buffer ELUTION.

- Purified DNA in the Elution Tube can be used immediately.
- Store the DNA at 4°C (short-term) or –20°C (long-term).

TROUBLESHOOTING

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.
LOW YIELD	
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	
Too much elution buffer used	Use less Buffer ELUTION.
SHEARED OR DEGRADED DNA	
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.
RNA CONTAMINATION	
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.

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Certified Quality-System mdc ISO 13485 ISO 9001

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