GenUP™ gDNA Kit

LOT: See product label EXPIRY DATE: See product label

ORDERING INFORMATION

PRODUCT	GenUP™ gDNA Kit		
CAT. NO.	BR0700601	BR0700602	BR0700603
SIZE	10 preps	50 preps	250 preps
COMPONENTS			
Buffer LYSIS LG	10 ml	25 ml	120 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)	5 vials (add 1.5 ml water)
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
Mini Filters (blue)	10	50	5 × 50
Collection Tubes (2 ml)	20	2 × 50	10 × 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

- Universal kit for DNA isolation from various starting materials
- Fast and simple procedure with >100 µg gDNA filter-binding capacity
- High yields of pure DNA

APPLICATIONS

- Isolation of genomic DNA from tissues, cells, rodent tail, buccal swaps and paraffin samples

DESCRIPTION

biotechrabbit GenUP™ gDNA Kit is designed for a fast and efficient genomic DNA isolation from various sources and different amounts of starting material, such as mammalian tissues (including paraffin-embedded), buccal swabs and eukaryotic cell cultures. After a few initial procedures, the genomic DNA is bound to a Mini Filter, washed and then eluted in a low-salt buffer. High yields of pure, unshared genomic DNA are ready to be used in all demanding molecular biology applications, including enzymatic reactions and sequencing.

SPECIFICATIONS

STARTING MATERIAL	Tissue samples (up to 40 mg), rodent tail specimens of 0.5–1 cm in length, eukaryotic cells (up to 5×10^6), formalin-fixed paraffin-embedded tissue samples (FFPE, 2–4 slices), buccal swabs
EXTRACTION TIME	Approximately 8 min after lysis
BINDING CAPACITY	>100 µg DNA
TYPICAL YIELD	Variable; approximately 65 µg
AVERAGE PURITY	A_{260}/A_{280} 1.7–2.0

MATERIALS SUPPLIED BY THE USER

- 96–99.8% ethanol
- Centrifugation tubes
- Pipet tips
- Double-distilled water
- *Optional:* RNase A (100 mg/ml)
- GenUP FFPE Paraffin Removal Solution (BR0701601, for paraffin embedded tissues)

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.

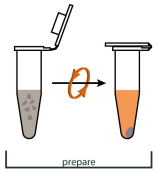
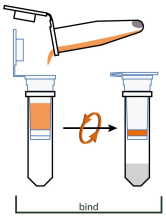
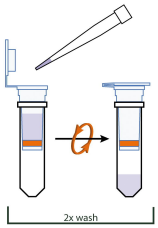
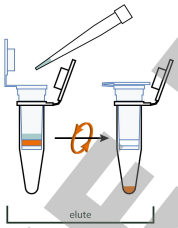
CAT. NO.	CONCENTRATE	ETHANOL	FINAL VOLUME
BR0700601	6 ml	14 ml	20 ml
BR0700602	24 ml	56 ml	80 ml
BR0700603	60 ml	140 ml	200 ml

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

BR0700601	0.3 ml
BR0700602, BR0700603	1.5 ml for 5 × 0.3 ml aliquots

- Mark all vials and filters to avoid confusion when purifying multiple preps.
- Heat thermomixer or water bath at 50°C (and 90°C for paraffin removal).
- Perform all centrifugation steps at room temperature.

SHORT PROTOCOL

STEPS	SCHEME
• Collect the sample material and perform lysis. • Centrifuge to pellet unlysed material. • Transfer the supernatant to a new tube and add Buffer BINDING BD.	
• Apply the mixture to a Mini Filter (blue) and centrifuge.	
• Wash the DNA twice with Buffer WASH C and centrifuge. • Centrifuge again to remove residual ethanol.	
• Elute DNA with Buffer ELUTION and centrifuge. • Purified genomic DNA in the Elution Tube is ready for use.	

PROTOCOL FOR ISOLATING GENOMIC DNA FROM TISSUE OR RODENT TAIL

PROCEDURE	NOTES
• Cut up to 40 mg tissue sample into small pieces and place in a 1.5 ml reaction tube.	
• Add 400 µl Buffer LYSIS LG and 25 µl Proteinase K. • *Optionally*, add 3 µl RNase A (100 mg/ml, not included) • Mix vigorously by pulse vortexing for 5 s. • Incubate at 50°C with shaking until the sample is completely lysed (approximately 0.5–2 h for tissue and approximately 3 h for rodent tails).	• 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. • The solution becomes clear when the lysis is complete. Incomplete lysis can decrease DNA yield.
• Centrifuge at 10,000 × *g* (12,000 rpm) for 30 s to pellet unlysed material. • Transfer the supernatant into a new 1.5 ml tube.	
• Add 200 µl Buffer BINDING BD to the lysed sample. • Mix by vortexing or by pipetting up and down several times.	• Insufficient homogenization can decrease DNA yield.
• Apply the sample to a Mini Filter (blue) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Collection Tube with the filtrate.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.
• Add 700 µl Buffer WASH C to the Mini Filter placed into a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection Tube.	• Before use, prepare Buffer WASH C as described above.
• Add 700 µl Buffer WASH C to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection Tube.	
• Centrifuge again for 2 min 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 6000 × *g* (8000 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 ISOLATING GENOMIC DNA FROM CELL CULTURES

PROCEDURE	NOTES
• Pellet up to 5×10^6 cells by centrifugation for 10 min at 5000 × *g* (7500 rpm).	
• Add 200 µl Buffer LYSIS LG and 25 µl Proteinase K. • *Optionally*, add 3 µl RNase A (100 mg/ml, not included) • Mix vigorously by pulse vortexing for 5 s. • Incubate at 50°C with shaking until the sample is completely lysed (approximately 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. • The solution becomes clear when the lysis is complete. Incomplete lysis can decrease DNA yield.
• Add 200 µl Buffer BINDING BD to the lysed sample. • Mix by vortexing or by pipetting up and down several times.	• Insufficient homogenization can decrease DNA yield.
• Apply the sample to a Mini Filter (blue) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Collection Tube with the filtrate.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.
• Add 700 µl Buffer WASH C to the Mini Filter placed into a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection Tube.	• Before use, prepare Buffer WASH C as described above.
• Add 700 µl Buffer WASH C to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection Tube.	
• Centrifuge again for 2 min 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 6000 × *g* (8000 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 ISOLATING GENOMIC DNA FROM PARAFFIN-EMBEDDED TISSUE

PROCEDURE	NOTES
• Cut FFPE slices from your FFPE sample with a microtome.	• Find full instructions in the manual of the GenUP FFPE Paraffin Removal Solution (BR0701601)
• Place 2–4 FFPE slices in a 1.5 ml reaction tube.	• Depending on the tissue and the age of the FFPE block, more slices are needed in the case of low recovery.
• Add 3–5 drops of GenUP FFPE Paraffin Removal Solution to the FFPE slices.	• The FFPE slices dissolve immediately. Add enough solution to submerge the FFPE slices (approximately 300–500 µl).
• Add 400 µl Buffer LYSIS LG and 25 µl Proteinase K. • *Optionally*, add 3 µl RNase A (100 mg/ml, not included) • Mix vigorously by pulse vortexing for 5 s. • Incubate at 50°C with shaking until the sample is completely lysed (approximately 0.5–2 h).	• 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. • The solution becomes clear when the lysis is complete. Incomplete lysis can decrease DNA yield.
• Incubate for an additional 10 min at 90°C. • Remove the reaction tube from the thermomixer.	• If the two phases have not separated, centrifuge the reaction mixture briefly to collect the aqueous phase at the bottom of the reaction tube.
• Transfer the lower colorless aqueous phase containing the DNA to a new 1.5 ml reaction tube. • Add 200 µl Buffer BINDING BD to the lysed sample. • Mix by vortexing or by pipetting up and down several times.	• The upper, orange phase contains the paraffin. • Insufficient homogenization can decrease DNA yield.
• Apply the sample to a Mini Filter (blue) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Collection Tube with the filtrate.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.
• Add 700 µl Buffer WASH C to the Mini Filter placed into a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection Tube.	• Before use, prepare Buffer WASH C as described above.
• Add 700 µl Buffer WASH C to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection Tube.	
• Centrifuge again for 2 min 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 6000 × *g* (8000 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 ISOLATING GENOMIC DNA FROM BUCCAL SWABS

PROCEDURE	NOTES
• Place the swab in a 1.5 ml reaction tube.	• The shaft of the swab can be removed so that tube can be closed.
• Add 400 µl Buffer LYSIS LG and 25 µl Proteinase K. • *Optionally*, add 3 µl RNase A (100 mg/ml, not included) • Mix vigorously by pulse vortexing for 5 s. • Incubate at 50°C with shaking until the sample is completely lysed (10–15 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. • The solution becomes clear when the lysis is complete. Incomplete lysis can decrease DNA yield.
• After the lysis is complete, remove the swab from the tube, and discard the swab.	• Squeeze the swab against the wall to maximize lysate volume in the tube.
• Add 200 µl Buffer BINDING BD to the lysed sample. • Mix by vortexing or by pipetting up and down several times.	• Insufficient homogenization can decrease DNA yield.
• Apply the sample to a Mini Filter (blue) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Collection Tube with the filtrate.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.
• Add 700 µl Buffer WASH C to the Mini Filter placed into a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection Tube.	• Before use, prepare Buffer WASH C as described above.
• Add 700 µl Buffer WASH C to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection Tube.	
• Centrifuge again for 2 min 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 6000 × *g* (8000 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	
Excessive starting material or insufficient lysis	Follow recommendations for the maximum amount of starting material and perform lysis until the solution becomes clear and viscosity decreases. Do not load cell debris onto Mini Filter. After lysis centrifuge the lysate to pellet unlysed material.
LOW YIELD	
Excessive starting material, insufficient lysis	Follow recommendations for the maximum amount of starting material and perform lysis until the solution becomes clear and viscosity decreases.
Incomplete elution	Increase the incubation time with Buffer ELUTION up to 5 min, or repeat the elution. Use the recommended volume of Buffer ELUTION.
Incorrect binding	After addition of Buffer BINDING BD, mix the sample carefully to ensure the mixture is a homogeneous mix.
LOW CONCENTRATION OF ELUTED DNA	
Too much of elution buffer used	Do not exceed the recommended volume of Buffer ELUTION. Perform the elution in two steps, each step using half of the elution volume. The first eluate typically has higher DNA concentration.
SHARED OR DEGRADED DNA	
Poor quality starting material	Use fresh material or ensure the material is immediately frozen in liquid nitrogen. Store at −80°C and avoid repeated freezing and thawing.
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 if required.

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 genomic DNA purification from tissue samples and subsequent analysis of purified genomic DNA in PCR target 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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