GenUP™ Bacteria gDNA Kit

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

PRODUCT	GenUP™ Bacteria gDNA Kit		
CAT. NO.	BR0700701	BR0700702	BR0700703
SIZE	10 preps	50 preps	250 preps
COMPONENTS			
Buffer LYSIS LB	15 ml	15 ml	60 ml
Buffer BINDING BG	16 ml	30 ml	120 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 A (concentrate)	5 ml (add 5 ml ethanol)	15 ml (add 15 ml ethanol)	70 ml (add 70 ml ethanol)
Buffer WASH C (concentrate)	6 ml (add 14 ml ethanol)	24 ml (add 56 ml ethanol)	60 ml (add 140 ml ethanol)
Buffer ELUTION	2 × 2 ml	15 ml	2 × 30 ml
Mini Filters (yellow ring)	10	50	5 × 50
Collection Tubes (2 ml)	40	4 × 50	20 × 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

- Fast and simple procedure
- gDNA from up to 1×10^9 bacteria
- High yields of pure DNA for demanding applications

APPLICATIONS

- Isolation of bacterial genomic DNA from Gram-positive and Gram-negative bacteria

DESCRIPTION

biotechrabbit™ GenUP Bacterial gDNA Kit has been specially developed for quick and easy purification of bacterial genomic DNA from both Gram-negative and difficult to process Gram-positive bacteria.
A combined lysozyme and proteolytic lysis steps allow efficient cell disruption. The DNA is bound to a high-capacity filter, washed and then eluted in a separate tube. The purified DNA is ready to be used in all demanding molecular biology applications, including PCR, enzymatic digestions, cloning and other.

SPECIFICATIONS

STARTING MATERIAL	1×10^9 Gram-positive or Gram-negative bacterial cells
EXTRACTION TIME	Approximately 45 min
BINDING CAPACITY	> 50 µg DNA
TYPICAL YIELD	Variable; approximately 35 µg

MATERIALS SUPPLIED BY THE USER

- 96–99.8 % ethanol
- Centrifugation tubes
- Pipet tips
- Double-distilled water
- TE Buffer
- Lysozyme solution (10 mg/ml in TE buffer)
- *Optional:* RNase A (10 mg/ml)

STEPS BEFORE STARTING

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

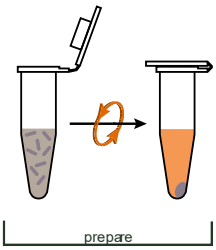
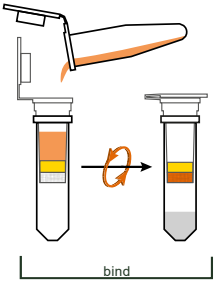
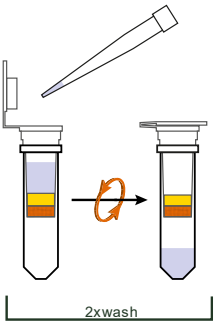
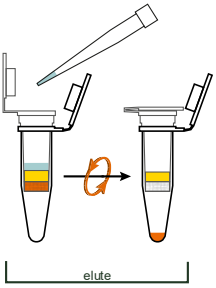
CAT. NO.		CONCENTRATE	ETHANOL	FINAL VOLUME
Buffer WASH A	BR0700701	5 ml	5 ml	10 ml
	BR0700702	15 ml	15 ml	30 ml
	BR0700703	70 ml	70 ml	140 ml
Buffer WASH C	BR0700701	6 ml	14 ml	20 ml
	BR0700702	24 ml	56 ml	80 ml
	BR0700703	60 ml	140 ml	200 ml

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

BR0700701	0.3 ml
BR0700702, BR0700703	1.5 ml for 5 × 0.3 ml aliquots

- Mark all vials and filters to avoid confusion when purifying multiple preps.
- Perform all centrifugation steps at room temperature.
- Heat thermomixer or water bath (37 °C and 50 °C).

SHORT PROTOCOL

STEPS	SCHEME
• Pellet 5.0–15.0 ml bacterial culture and centrifuge. Remove the supernatant. • Resuspend the cell pellet in TE buffer. • Add lysozyme and incubate. • Add Buffer LYSIS LB and Proteinase K, mix and incubate. • Centrifuge to pellet unlysed material	
• Transfer the lysate to a new tube and add Buffer BINDING BG. • Apply the mixture to a Mini Filter and centrifuge.	
• Wash with Buffer WASH A and Buffer WASH C and centrifuge. • Centrifuge again to remove residual ethanol.	
• Elute DNA in Buffer ELUTION. • Purified DNA in the Elution Tube is ready for use.	

PROTOCOL FOR ISOLATING GENOMIC DNA FROM BACTERIAL CELLS

PROCEDURE	NOTES
• Transfer 1×10^9 Gram-positive or Gram-negative bacterial cells to a 15 ml reaction tube. • Centrifuge at maximum speed for 1 min to pellet the cells. • Remove the supernatant completely and discard.	• Completely removing the supernatant improves performance in downstream applications.
• Resuspend the cell pellet in 200 µl TE buffer. • Add 15 µl lysozyme solution (10 mg/ml in TE buffer, not included in the kit) and mix by pulse vortexing. • Incubate at 37 °C until the sample becomes clear.	• Incubation time for lysis depends on the bacteria strain. Gram-positive bacteria require longer lysis time than Gram-negative. • Continuously shaking is recommended.
• Add 200 µl Buffer LYSIS LB and 25 µl Proteinase K. • *Optionally*, add 3 µl RNase A (10 mg/ml, not included in the kit). • Mix vigorously by pulse vortexing for 5 s. • Incubate at 50 °C until the sample is completely lysed (approximately 15 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.
• Centrifuge at 10,000 × *g* (12,000 rpm) for 30 s to spin down unlysed material. • Transfer the supernatant to a new 1.5 ml tube.	• This step can be skipped if all material is completely lysed.
• Add 400 µl Buffer BINDING BG. • Mix by vortexing or by pipetting up and down several times.	• For improved yield, mix thoroughly.
• Transfer the sample to a Mini Filter (yellow ring) located 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 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 500 µl Buffer WASH A 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 750 µl Buffer WASH C. • 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. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min to remove residual ethanol. • Discard the Collection Tube.	

- Place the Mini Filter into an Elution Tube.
- Add 50–100 µl Buffer ELUTION into the center of the Mini Filter.
- Incubate at room temperature for 1 min.
- To improve yield, perform elution twice using ½ volume of Buffer ELUTION.

- 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).

TROUBLESHOOTING

PROBLEM	SOLUTION
CLOGGED MINI FILTER	
Too much starting material or insufficient lysis	Follow recommendations for the maximum amount of starting material and perform lysis until the solution becomes clear and viscosity is reduced
LOW YIELD	
Too much starting material or insufficient lysis	Follow recommendations for the maximum amount of starting material and perform lysis until the solution becomes clear and viscosity is reduced
Incomplete elution	Increase the elution time up to 5 min, or repeat the elution. Use the recommended volume of Buffer ELUTION.
Incorrect mixing of Buffer BINDING BG	After addition of Buffer BINDING BG, mix the sample carefully to ensure the mixture is 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 and avoid repeated freezing and thawing.
RNA CONTAMINATION	
No RNase treatment	The treatment with RNase is optional. If the 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!
- Buffer WASH A contains guanidine isothiocyanate, which is harmful to health. Contact with acids liberates very toxic gas!

CERTIFICATE OF ANALYSIS

The components of the kit were tested for isolation of bacterial genomic DNA from bacterial cells and subsequent analysis of purified 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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Certified Quality-System mdc ISO 13485 ISO 9001

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LIMITATION OF USE

For Research Use Only. biotechrabbit does not assume any warranty regarding the design, merchantability or fitness for a particular purpose of the product, especially, but not limited to, for any *in vitro* diagnostic or other usages of the products. It is the sole responsibility of the customer to obtain the required approvals from the competent authorities. The product is not suitable for administration to humans or animals.

valid from 24.08.2016