biotechrabbit
leap and lead

GenUP™ Plasmid Kit

LOT: See product label   EXPIRY DATE: See product label

ORDERING INFORMATION

PRODUCT	GenUP™ Plasmid Kit		
CAT. NO.	BR0700201	BR0700202	BR0700203
SIZE	10 preps	50 preps	250 preps
COMPONENTS			
Buffer RESUSPENSION	12 ml	30 ml	150 ml
Buffer LYSIS LP	15 ml	30 ml	150 ml
Buffer NEUTRALIZATION	12 ml	32 ml	200 ml
Buffer WASH PA (ready-to-use)	15 ml	30 ml	170 ml
Buffer WASH PB (concentrate)	6 ml (add 9 ml ethanol)	20 ml (add 30 ml ethanol)	80 ml (add 120 ml ethanol)
Buffer ELUTION EP	2 ml	15 ml	30 ml
Mini Filters (orange)	10	50	5 × 50
Collection Tubes (2 ml)	10	50	5 × 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.

FEATURES

- Universal kit for both high- and low-copy-number plasmids isolation
- Fast and simple procedure
- High yields of pure plasmid DNA suitable for all applications

APPLICATIONS

- Plasmid DNA isolation from Gram-negative and Gram-positive bacteria

DESCRIPTION

biotechrabbit™ GenUP Plasmid Kit is designed for fast and efficient plasmid purification from 0.5–15 ml bacterial suspensions in a mini format. After performing an alkaline lysis step and precipitating chromosomal DNA and bacterial proteins, plasmid DNA is bound to a Mini Filter, washed and then eluted in a low-salt buffer. Typically, yields are up to 60 µg plasmid DNA from a 15 ml bacterial suspension, depending on a plasmid copy number. The purified plasmid DNA is ready to be used in all demanding molecular biology applications, including enzymatic reactions and sequencing.

SPECIFICATIONS

STARTING MATERIAL	Bacterial cultures: 0.5–15 ml for high-copy-number plasmids, low-copy-number plasmids or cosmids
EXTRACTION TIME	Approximately 15 min
BINDING CAPACITY	Approximately 80 µg plasmid DNA
TYPICAL YIELD	Variable: 45–60 µg high-copy plasmid DNA is typically purified from 15 ml culture, or 6–20 µg high-copy plasmid DNA from 2 ml culture
AVERAGE PURITY	A_{260}/A_{280} 1.7–2.0

MATERIALS SUPPLIED BY THE USER

- 96–99.8% ethanol
- Reaction tubes
- Pipet tips
- *Optional:* for Gram-positive bacteria
  - STE buffer (6.7% saccharose, 50 mM Tris-HCl, pH 8.0, 1 mM EDTA)
  - Lysozyme
  - Mutanolysin for difficult to lyse Gram positive bacteria

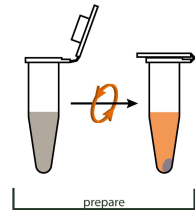
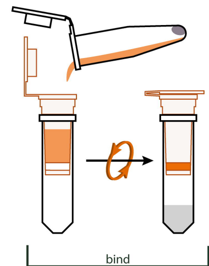
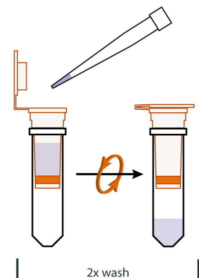
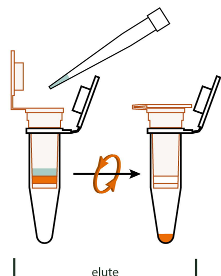
STEPS BEFORE STARTING

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

CAT. NO.	CONCENTRATE	ETHANOL	FINAL VOLUME
BR0700201	6 ml	9 ml	15 ml
BR0700202	20 ml	30 ml	50 ml
BR0700203	80 ml	120 ml	200 ml

- Mark all vials and filters to avoid confusion when purifying multiple preps.
- Perform all centrifugation steps at room temperature.
- Resolve white precipitates in Buffer LYSIS LP by gentle heating at 30–40°C. Then let Buffer LYSIS LP cool down to room temperature again.

SHORT PROTOCOL

STEPS	SCHEME
• Pellet bacterial culture and centrifuge. Remove the supernatant. • Resuspend the cell pellet in Buffer RESUSPENSION and vortex. • *Optional:* Pre-treatment of Gram-positive bacteria with lysozyme. • Add Buffer LYSIS LP, mix and incubate. • Add Buffer NEUTRALIZATION, mix and centrifuge.	
• Transfer lysate to Mini Filter (orange) and centrifuge.	
• Wash with Buffer WASH PA and Buffer WASH PB and centrifuge. • Centrifuge again to remove residual ethanol.	
• Elute DNA with Buffer ELUTION EP. • Purified DNA in the Elution Tube is ready for use.	

PROTOCOL FOR ISOLATING HIGH-COPY-NUMBER PLASMID DNA FROM 0.5–5 ML BACTERIAL CULTURE

PROCEDURE	NOTES
• Transfer 0.5–5 ml overnight bacterial culture to a centrifuge tube. • Centrifuge for 1 min at maximum speed to pellet the cells. • Remove the supernatant completely and discard.	• Completely removing the supernatant improves performance in downstream applications.
• Resuspend the pellet in 250 µl Buffer RESUSPENSION and mix by vortexing or pipetting up and down.	• Ensure complete resuspension of the pellet.
• Add 250 µl Buffer LYSIS LP, and mix by inverting the tube seven times. • Incubate at room temperature for 5 min. Invert the tube few more times during incubation for improved lysis.	• Do not vortex. This will shear chromosomal DNA and leads to contamination of plasmid DNA. • Do not incubate longer than 5 min.
• Add 350 µl Buffer NEUTRALIZATION. • Mix gently but thoroughly by inverting seven times.	• Insufficient mixing can decrease yield.
• Centrifuge at maximum speed for 8 min to precipitate proteins and large chromosomal DNA.	
• Transfer the clarified supernatant to a Mini Filter (orange) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection tube.	• If the solution does not pass completely through the Mini Filter, repeat the centrifugation or use a longer centrifugation time.
• Add 500 µl Buffer WASH PA to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection tube.	
• Add 700 µl Buffer WASH PB. • 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 PB as described above.
• Centrifuge at maximum speed for 2 min to remove residual ethanol. • Discard the Collection Tube.	
• Place the Mini Filter in a new Elution Tube. • Add 50–100 µl Buffer ELUTION EP 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 EP. • Discard the Mini Filter only when you are sure that an additional elution is not required. • Using lower volumes of Buffer ELUTION EP increases final pDNA concentration.
• Purified plasmid DNA in the Elution Tube can be used immediately.	• Store the DNA at 4 °C (short-term) or −20 °C (long-term).

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PROTOCOL FOR ISOLATING HIGH- OR LOW-COPY-NUMBER PLASMID DNA FROM 5–15 ML BACTERIAL CULTURES

PROCEDURE	NOTES
• Transfer 5–15 ml overnight bacterial culture to a 15 ml reaction tube. • Centrifuge for 1 min at maximum speed to pellet the cells. • Remove the supernatant completely and discard.	• Completely removing the supernatant improves performance in downstream applications.
• Resuspend the pellet in 550 µl Buffer RESUSPENSION by vortexing or pipetting up and down. • Transfer the solution into a 2 ml reaction tube	• Ensure complete resuspension of the pellet.
• Add 550 µl Buffer LYSIS LP, and mix by inverting the tube seven times. • Incubate at room temperature for 5 min. Invert the tube few more times during incubation for improved lysis.	• Do not vortex. This will shear chromosomal DNA and leads to contamination of plasmid DNA. • Do not incubate longer than 5 min.
• Add 750 µl Buffer NEUTRALIZATION. • Mix gently but thoroughly by inverting seven times.	• Insufficient mixing can decrease yield.
• Centrifuge at maximum speed for 10 min to precipitate proteins and large chromosomal DNA.	
• Transfer 850 µl of the clarified supernatant to a Mini Filter (orange) placed in a Collection Tube. • Centrifuge at 10,000 × g (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection tube.	• If the solution does not pass completely through the Mini Filter, repeat the centrifugation or use a longer centrifugation time.
• Transfer the rest of the supernatant to the Mini filter. • Centrifuge, discard filtrate and re-use the Collection tube.	Repeat the last step using the same Mini Filter (orange).
• Add 650 µl Buffer WASH PA to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and reuse the Collection tube.	
• Add 750 µl Buffer WASH PB. • 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 PB as described above.
• Centrifuge at maximum speed for 2 min to remove residual ethanol. Discard the Collection Tube.	
• Add 100 µl Buffer ELUTION EP to the center of the Mini Filter placed in a new Elution Tube. • 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 EP. • Discard the Mini Filter only when you are sure that an additional elution is not required. • Using lower volumes of Buffer ELUTION EP increases final pDNA concentration.
• Purified plasmid 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 PLASMID DNA FROM GRAM-POSITIVE BACTERIA

PROCEDURE	NOTES
• Transfer 0.5–5 ml overnight bacterial culture to a centrifuge tube. • Centrifuge for 1 min at maximum speed to pellet the cells. • Remove the supernatant completely and discard.	• Completely removing the supernatant improves performance in downstream applications.
• Resuspend the cell pellet in 500 µl STE buffer and mix by vortexing or pipetting up and down. • Centrifuge for 1 min at maximum speed to pellet the cells. • Remove the supernatant completely and discard.	• Before use, prepare STE buffer (not provided) as described above. • Ensure complete resuspension of the pellet.
• Resuspend the cell pellet in 250 µl STE buffer containing 10 mg/ml lysozyme. • Incubate at 37 °C for 1–2 h.	• For lactobacillus strains that are difficult to lyse (e.g., LTH677), add 20–100 U/ml mutanolysin to the STE buffer.
• Add 250 µl Buffer LYSIS LP, and mix by inverting the tube five times. • Incubate at room temperature for 5 min. Invert the tube few more times during incubation for improved lysis.	• Do not vortex. This will shear chromosomal DNA and leads to contamination of plasmid DNA. • Do not incubate longer than 5 min.
• Add 350 µl Buffer NEUTRALIZATION. • Mix gently but thoroughly by inverting five times. • Centrifuge at maximum speed for 10 min to precipitate proteins and large chromosomal DNA.	• Insufficient mixing can decrease yield.
• Transfer the clarified supernatant to a Mini Filter (orange) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection tube.	• If the solution does not pass completely through the Mini Filter, repeat the centrifugation or use a longer centrifugation time.
• Add 500 µl Buffer WASH PA to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection tube.	
• Add 700 µl Buffer WASH PB. • 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 PB as described above.
• Centrifuge at maximum speed for 2 min to remove residual ethanol. • Discard the Collection Tube.	
• Add 50–100 µl Buffer ELUTION EP to the center of the Mini Filter placed in a Collection Tube. • 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 EP. • Discard the Mini Filter only when you are sure that an additional elution is not required.
• Purified plasmid 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
LOW YIELD	
Incorrect washing solution	Prepare the Buffer WASH PB as described above. Ensure the cap is closed tightly before storage.
Poor elution	Add Buffer ELUTION EP directly to the center of the Mini Filter membrane, even when using a small elution volume. Two elution steps result in better yields than one elution step.
Inefficient resuspension or lysis of bacterial cells	Ensure the cell pellet is completely resuspended. After the addition of Buffer LYSIS LP, the solution should become clear.
Incorrect neutralization	Do not shake or vortex the sample during neutralization. Invert the tube five times.
PROBLEMS WITH DOWNSTREAM APPLICATIONS (E.G., LIGATION)	
Salt carried over to elution	Follow carefully the instructions for the wash steps.
Ethanol carried over to elution	Follow carefully the instructions for the centrifugation after completing the wash steps. If the sample smells of ethanol, the centrifugation time can be extended.
Nicked plasmid DNA	Do not shake vigorously during lysis. Do not perform lysis longer than 5 min.

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 plasmid DNA purification and subsequent analysis of purified plasmid.

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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