GenUP™ Blood DNA Kit

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

PRODUCT	GenUP™ Blood DNA Kit		
CAT. NO.	BR0701301	BR0701302	BR0701303
SIZE	10 preps	50 preps	250 preps
COMPONENTS			
Buffer LYSIS LC	12 ml	25 ml	120 ml
Buffer BINDING BL	8 ml	50 ml	250 ml
Proteinase K (lyophilized)	1 vial (add 0.3 ml water)	2 vials (add 1.5 ml water)	6 vials (add 1.5 ml water)
Buffer WASH WA (ready-to-use)	8 ml	30 ml	120 ml
Buffer WASH WB (concentrate)	2 ml (add 18 ml ethanol)	10 ml (add 90 ml ethanol)	2 × 18 ml (add 162 ml ethanol)
Buffer ELUTION	2 × 2 ml	15 ml	2 × 30 ml
Mini Filters (red)	10	50	5 × 50
Collection Tubes (2.0 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

- Fast and simple procedure
- Genomic gDNA from fresh and frozen, EDTA- or citrate-treated blood
- Excellent genomic DNA quality in yields of up to 30 µg

APPLICATIONS

- Isolation of genomic DNA from up to 400 µl whole blood

DESCRIPTION

biotechrabbit™ GenUP Blood DNA Kit is designed for fast isolation of genomic DNA from up to 400 µl whole blood from fresh or frozen samples that have been stabilized with EDTA or citrate. After an efficient lysis step, genomic DNA is bound to a Mini Filter, washed and eluted. The isolation chemistry and extraction protocol are optimized for maximum yield. Including lysis, isolated DNA is available in approximately 24 min. The isolated DNA is suitable for a wide range of different molecular biology applications.
Protocols are available for isolating DNA from 200 µl or 400 µl whole blood samples.
The GenUP Blood DNA Kit is designed for the use with blood. For other starting material, such as cell-free body fluids (including cerebrospinal fluid, serum, plasma or urine), tissue, stool samples, buffy coat, cultured or isolated cells, swabs, dried blood spots, viruses, fungi, bacteria or parasites, please refer to the GenUP gDNA Kit (cat. no. BR0700601), GenUP Bacteria gDNA Kit (cat. no. BR0700701), GenUP Plant DNA Kit (cat. no. BR0700801) or GenUP Virus DNA/RNA Kit (cat. no. BR0701101).

SPECIFICATIONS

STARTING MATERIAL	Fresh or frozen whole blood; stabilized with EDTA or citrate (200 µl or 400 µl)
EXTRACTION TIME	Approximately 24 min
BINDING CAPACITY	>60 µg DNA
TYPICAL YIELD	Variable depending on the starting material; approximately 30 µg DNA
AVERAGE PURITY	A_{260}/A_{280} 1.7–2.0

MATERIALS SUPPLIED BY THE USER

- Phosphate buffered solution (PBS)
- 96–99.8% ethanol
- Centrifugation tubes
- 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 WB, close firmly, mix thoroughly and store at room temperature.

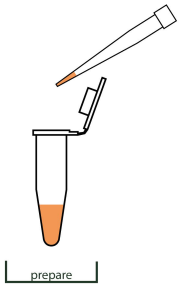
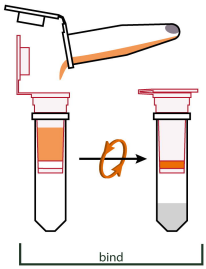
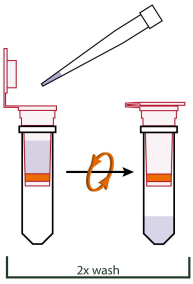
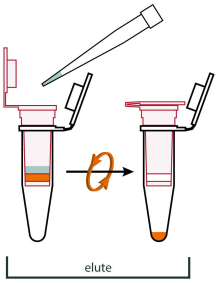
CAT. NO.	CONCENTRATE	ETHANOL	FINAL VOLUME
BR0701301	2 ml	18 ml	20 ml
BR0701302	10 ml	90 ml	100 ml
BR0701303	18 ml	162 ml	180 ml

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

BR0701301	0.3 ml
BR0701302, BR0701303	1.5 ml for 5 × 0.3 ml aliquots

- Mark all vials and filters to avoid confusion when purifying multiple preps.
- Centrifugation steps should be carried out at room temperature.
- Heat thermal mixer or water bath to 60°C.
- Warm Buffer ELUTION to 60°C.

SHOT PROTOCOL

STEPS	SCHEME
• If necessary, add PBS to the samples for a volume of 200 µl or 400 µl. • Add Buffer LYSIS LC and Proteinase K and incubate. • Add Buffer BINDING BL and mix well.	
• Transfer the sample to the Mini Filter (red) placed in a Collection Tube and centrifuge.	
• Add Buffer WASH WA and centrifuge. • Add Buffer WASH WB and centrifuge. • Centrifuge again to remove the wash buffer.	
• Elute DNA with Buffer ELUTION and centrifuge. • Purified DNA in the Elution Tube is ready for use.	

PROTOCOL FOR DNA ISOLATION FROM 200 µL WHOLE BLOOD

PROCEDURE	NOTES
• Transfer up to 200 µl whole blood to a 1.5 ml or 2 ml reaction tube. • Bring the volume to 200 µl with PBS, if necessary. • Add 200 µl Buffer LYSIS LC and 20 µl Proteinase K, mix vigorously by pulse vortexing for 10 s. • Incubate at 60°C for 10 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.
• *Optionally,* add 4 µl RNase A (100 mg/ml, not included in the kit), mix vigorously by pulse vortexing for 5 s. • Incubate 5 min at room temperature.	• If RNA is present in the sample, DNA and RNA are copurified. • This step can be skipped if RNA-free DNA is not required.
• *Optionally,* centrifuge 10 s to remove condensate from the lid of the tube.	
• Add 350 µl Buffer BINDING BL to the lysed sample. • Mix carefully by pipetting up and down 3–4 times.	• *Important:* Mix well but do not vortex, as vortexing reduces yield of DNA.
• Transfer the sample to a Mini Filter (red) placed in a Collection Tube. • Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 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.
• Place the Mini Filter into a new Collection Tube. • Add 400 µl Buffer WASH WA. • Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	
• Place the Mini Filter into a new Collection Tube. • Add 600 µl Buffer WASH WB. • Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection Tube.	• Before use, prepare Buffer WASH WB as described above.
• Add 600 µl Buffer WASH WB to the Mini Filter. • Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	
• Place the Mini Filter into a new Collection Tube. • Centrifuge at maximum speed for 3 min to remove all traces of ethanol. • Discard the Collection Tube.	
• Place the Mini Filter into an Elution Tube. • Add 200 µl Buffer ELUTION. • Incubate at room temperature for 2 min. • Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 min. • Discard the Mini Filter.	• Before use, ensure the Buffer ELUTION is warmed to 60°C. • 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 DNA ISOLATION FROM 400 µL WHOLE BLOOD

PROCEDURE	NOTES
• Transfer up to 400 µl whole blood to a 1.5 ml or 2 ml reaction tube. • Bring the volume to 400 µl with PBS, if necessary. • Add 400 µl Buffer LYSIS LC and 30 µl Proteinase K, mix vigorously by pulse vortexing for 10 s. • Incubate at 60 °C for 10 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.
• *Optionally,* add 4 µl RNase A (100 mg/ml, not included in the kit), mix vigorously by pulse vortexing for 5 s. • Incubate 5 min at room temperature.	• If RNA is present in the sample, DNA and RNA are copurified. • This step can be skipped if RNA-free DNA is not required.
• *Optionally,* centrifuge 10 s to remove condensate from the lid of the tube.	
• Add 700 µl Buffer BINDING BL to the lysed sample. • Mix carefully by pipetting up and down 3–4 times.	• *Important:* Mix well but do not vortex, as vortexing reduces yield of DNA.
• Transfer 750 µl sample to a Mini Filter (red) placed in a Collection Tube. • Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 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.
• Transfer the remainder of the sample to the Mini Filter placed in a new Collection Tube. • Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 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.
• Place the Mini Filter into a new Collection Tube. • Add 400 µl Buffer WASH WA. • Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	
• Place the Mini Filter into a new Collection Tube. • Add 600 µl Buffer WASH WB. • Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection Tube.	• Before use, prepare Buffer WASH WB as described above.
• Add 600 µl Buffer WASH WB to the Mini Filter. • Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	
• Place the Mini Filter into a new Collection Tube. • Centrifuge at maximum speed for 3 min to remove all traces of ethanol. • Discard the Collection Tube.	
• Place the Mini Filter into an Elution Tube.	• Before use, ensure the Buffer ELUTION is

- Add 200 µl Buffer ELUTION.
- Incubate at room temperature for 2 min.
- Centrifuge at 11,000 ×*g* (~12,000 rpm) for 1 min.
- Discard the Mini Filter.

warmed to 60°C.
- 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.
LOW YIELD	
Insufficient lysis	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 BL	Mix the sample with Buffer BINDING BL by pipetting or vortexing before transferring to the Mini Filter.
LOW DNA CONCENTRATION	
Too much Buffer ELUTION 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 genomic DNA purification from whole blood samples and subsequent spectrophotometrically measurements, gel electrophoresis and 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

valid from 24.08.2016