GenUP™ Blood RNA Kit

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

PRODUCT	GenUP™ Blood RNA Kit		
CAT. NO.	BR0701401	BR0701402	BR0701403
SIZE	10 preps	50 preps	250 preps
COMPONENTS			
Buffer LYSIS LE (concentrate)	30 ml (add 970 ml water)	30 ml (add 970 ml water)	4 × 30 ml (add 970 ml water)
Buffer LYSIS LR	15 ml	30 ml	160 ml
Buffer WASH A (concentrate)	5 ml (add 5 ml ethanol)	15 ml (add 15 ml ethanol)	70 ml (add 70 ml ethanol)
Buffer WASH B (concentrate)	6 ml (add 24 ml ethanol)	16 ml (add 64 ml ethanol)	36 ml (add 144 ml ethanol)
Water, RNase-free (for ELUTION)	2 ml	2 × 2 ml	15 ml
Mini Filters DNA (blue)	10	50	5 × 50
Mini Filters RNA (violet)	10	50	5 × 50
Collection Tubes (2.0 ml)	60	6 × 50	30 × 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 Buffer LYSIS LE at 4°C after water was added.

FEATURES

- Fast and simple procedure
- High quality RNA isolated from fresh and frozen, EDTA- or citrate-treated blood
- Physical removal of DNA, no DNase treatment, no toxic β-mercaptoethanol

APPLICATIONS

- Isolation of up to 8 µg total RNA from 0.5–1.0 ml whole blood

DESCRIPTION

biotechrabbit™ GenUP Blood RNA Kit is designed for fast isolation of total RNA from up to 1 ml whole blood from fresh or frozen samples that have been stabilized with EDTA or citrate. Erythrocytes are removed in an initial lysis step. After an second lysis step, genomic DNA is bound to a Mini Filter DNA, which can be discarded. RNA is selectively bound to a Mini Filter RNA, washed with two different buffers and eluted. Including lysis, isolated RNA is available in approximately 45 min. The isolated RNA is suitable for a wide range of different molecular biology applications, including RT-PCR.
The GenUP Blood RNA 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 Total RNA Kit (BR07009), GenUP Virus RNA Kit (BR07010), GenUP Virus DNA/RNA Kit (BR07011) or UPzol RNA Isolation Solution (BR07001).

SPECIFICATIONS

STARTING MATERIAL	Fresh whole blood; stabilized with EDTA or citrate (0.5–1.0 ml)
EXTRACTION TIME	Approximately 45 min
BINDING CAPACITY	>20 µg RNA
TYPICAL YIELD	Variable depending on the starting material; 1–8 µg RNA

MATERIALS SUPPLIED BY THE USER

- 96–99.8% ethanol
- Double-distilled water
- 15 ml tubes (“Falcon Tubes”)
- 1 l bottle for Buffer LYSIS LE (concentrate)
- Pipet tips
- *Optional:* DNase I
- Crushed ice for chilling reagents

STEPS BEFORE STARTING

- Add the following volume of 96–99.8% ethanol to the indicated buffer bottles, close firmly, mix thoroughly and store at room temperature.

CAT. NO.		CONCENTRATE	ETHANOL	FINAL VOLUME
Buffer WASH A	BR0701401	5 ml	5 ml	10 ml
	BR0701402	15 ml	15 ml	30 ml
	BR0701403	70 ml	70 ml	140 ml
Buffer WASH B	BR0701401	6 ml	24 ml	30 ml
	BR0701402	16 ml	64 ml	80 ml
	BR0701403	36 ml	144 ml	180 ml

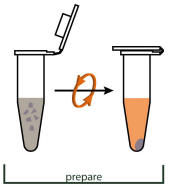
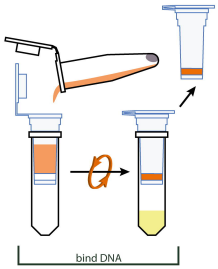
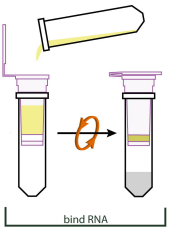
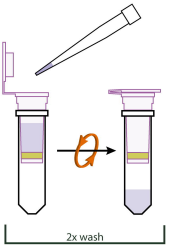
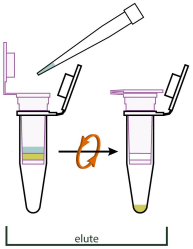
- Transfer the content of the Buffer LYSIS LE (concentrate) bottle to a 1 l bottle labeled "Buffer LYSIS LE". Add 970 ml double-distilled water, mix well and store at 4°C.
- Do not freeze blood samples before use.
- Centrifugation steps should be carried out at room temperature or 4°C, as indicated.
- Mark all vials and filters to avoid confusion when purifying multiple preps.

GUIDELINES FOR PREVENTION OF RNA DEGRADATION

Special care should be taken to minimize contamination with RNases, as RNA is extremely sensitive to degradation.

- Always wear gloves, and change them frequently.
- Keep all tubes closed when possible.
- Keep samples and isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free).
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All glassware should be treated before use to ensure that it is RNase-free.
  - Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four or more hours before use. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware.
  - Autoclaving alone will not inactivate many RNases completely. The glassware should be immersed in 0.1% diethylpyrocarbonate (DEPC) solution for 12 h at 37°C before autoclaving or heating to 100°C for 15 min to remove residual DEPC.
- Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers must be prepared with DEPC-treated RNase-free double-distilled water.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

SHORT PROTOCOL

STEPS	SCHEME
• Lyse erythrocytes two times by adding Buffer LYSIS LC, incubation and centrifugation. • Homogenize and lyse remaining RNA containing cells by adding Buffer LYSIS LR, incubate and centrifuge.	
• Transfer the lysate to the Mini Filter DNA (blue). • Centrifuge, and discard the Mini Filter DNA.	
• Add equal volume 70% ethanol and transfer the filtrate to the Mini Filter RNA (violet). • Centrifuge.	
• Add Buffer WASH A and centrifuge. • Add Buffer WASH B and centrifuge. • Centrifuge once more to remove residual ethanol.	
• Elute RNA with Water, RNase-free (for ELUTION) and centrifuge. • Purified RNA in the Elution Tube is ready for use.	

PROTOCOL FOR ISOLATING RNA FROM 0.5–1.0 ML WHOLE BLOOD

PROCEDURE	NOTES
• Transfer 0.5–1.0 ml fresh blood to a 15 ml tube. Add 10 ml chilled Buffer LYSIS LE. • Mix by vortexing briefly. • Incubate on ice for 15 min, vortexing twice during the incubation.	• If the expected amount of leukocytes is >1×10^7, reduce the sample size accordingly. • Thaw frozen blood samples before use. • Before use, prepare Buffer LYSIS LE as described above and chill to 4°C.
• Centrifuge at 2500 × *g* (3000 rpm) for 5 min at 4°C. • Discard the supernatant completely. • Invert the tube on a paper towel to drain residual supernatant.	• Do not discard the pellet. • Traces of the supernatant reduce the purification efficiency.
• Add 5 ml Buffer LYSIS LE to the cell pellet. • Resuspend the cell pellet by shaking vigorously by hand.	• Ensure Buffer LYSIS LE has been chilled to 4°C.
• Centrifuge at 2500 × *g* (3000 rpm) for 5 min at 4°C. • Discard the supernatant completely. • Invert the tube on a paper towel to drain residual supernatant.	• Do not discard the pellet. • Traces of the supernatant reduce the purification efficiency.
• Add 600 µl Buffer LYSIS LR and incubate for 2 min at room temperature. • Resuspend the cell pellet completely by pipetting up and down.	• For maximum yield, ensure the cells are completely resuspended and no clumps are visible. If necessary, incubate additional 2 min.
• Transfer the lysed sample to a Mini Filter DNA (blue) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Mini Filter DNA.	• Do not discard the filtrate, as it contains the RNA. • If the solution has not completely passed through the Mini Filter DNA, centrifuge again at higher speed or prolong the centrifugation time.
• Add an equal volume of 70% ethanol (600 µl) to the filtrate and mix by pipetting.	
• Transfer 650 µl sample to the Mini Filter RNA (violet) placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Collection Tube with the filtrate.	
• Transfer the remaining sample to the Mini Filter RNA placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 2 min. • Discard the Collection Tube with the filtrate.	
• Place the Mini Filter RNA into a new Collection Tube. • Add 500 µl Buffer WASH A. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH A as described above.

- Place the Mini Filter RNA into a new Collection Tube.
- Add 700 µl Buffer WASH B.
- Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min.
- Discard the Collection Tube with the filtrate.

- Place the Mini Filter RNA into a new Collection Tube.
- Centrifuge at maximum speed for 3 min to remove residual ethanol.
- Discard the Collection Tube.

- Place the Mini Filter RNA into a new Elution Tube.
- Add 30–50 µl Water, RNase-free (for ELUTION).
- Incubate at room temperature for 1 min.
- Centrifuge at 6000 × *g* (8000 rpm) for 1 min.
- Discard the Mini Filter RNA.
- Different volumes of RNase-free water can be used (minimum 20 µl).

- Purified RNA in the Elution Tube can be used immediately.
- Store the RNA at 4°C (short-term) or −80°C (long-term).

TROUBLESHOOTING

PROBLEM	SOLUTION
CLOGGED SPIN FILTER	
Insufficient disruption or homogenization	Reduce the amount of starting material. After lysis, centrifuge lysate to pellet debris and continue the protocol using the supernatant.
LOW YIELD	
Insufficient disruption or homogenization	Reduce the amount of starting material. Do not overload the Spin Filter DNA.
Incomplete elution	Increase the elution time up to 5 min, or repeat the elution. Use a higher elution volume or elute in two steps.
DNA CONTAMINATION	
Too much starting material	Reduce the amount of starting material.
Incorrect lysis of starting material	Use the recommended technique to lyse the cell pellet. Perform an on-filter DNase digestion after binding RNA to the Mini Filter RNA. Alternatively, perform DNase digestion on the eluate.
TOTAL RNA DEGRADED	
RNA source inappropriately handled or stored	Ensure that the starting material is fresh. Ensure that the protocol — especially the first steps — has been performed quickly.
RNase contamination	Use sterile, RNase-free filter tips. Ensure pipets, devices and workplace are clean. Wear gloves.
PURIFIED RNA DOES NOT PERFORM WELL IN DOWNSTREAM APPLICATIONS (E.G., RT-PCR)	
Ethanol carried over to elution	Increase the centrifugation time when removing ethanol.
Salt carried over to elution	Ensure that Buffer WASH A and Buffer WASH B are at room temperature. Dissolve precipitates in the washing solutions by warming carefully.

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!
- Buffers LYSIS LR and WASH A contain guanidine isothiocyanate, which is harmful to health. Contact with acids liberates very toxic gas!

QUALITY CONTROL

The components of the kit were tested for total RNA isolation from whole blood samples and subsequent analysis.

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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