GenUP™ Micro RNA Kit

LOT: See product label EXPIRY DATE: See product label

ORDERING INFORMATION

PRODUCT	GenUP™ Micro RNA Kit		
CAT. NO.	BR0701901	BR0701902	BR0701903
SIZE	10	50	250
COMPONENTS			
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	3 × 2 ml	25 ml
Mini Filters DNA (blue)	10	50	5 × 50
Mini Filters RNA (violet)	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.

FEATURES

- Fast and simple procedure
- Optimized binding conditions for high yields of small RNAs
- Physical removal of DNA, no DNase treatment, no toxic β-mercaptoethanol

APPLICATIONS

- Efficient isolation of snRNA, miRNA, siRNA, tRNA, rRNA and mRNA from various starting material

DESCRIPTION

biotechrabbit™ GenUP Micro RNA Kit is designed for high yields of small RNA molecules and total RNA, including snRNA, miRNA, siRNA, tRNA, rRNA and mRNA, without the use of highly toxic β-mercaptoethanol. After using well established filter-technology to selectively remove genomic DNA, the RNA is bound, washed and eluted from the filter membrane using RNase-free water.

SPECIFICATIONS

STARTING MATERIAL	Eukaryotic cells (5×10^6) Fresh or frozen tissue or biopsies (up to 20 mg) Gram-positive and Gram-negative bacteria (up to 1×10^9)
EXTRACTION TIME	Typically 15–40 minutes
BINDING CAPACITY	100 µg
YIELD	Depends on the type and the amount of the starting material
RECOVERY RATE	High rate of recovery for small RNA molecules

MATERIALS SUPPLIED BY THE USER

- 96–99.8% ethanol
- Isopropanol
- Reaction tubes
- DNase I, optional
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0, for bacteria)
- Lysozyme (50 mg/ml in water, store aliquots at −20°C)

STEPS BEFORE STARTING

- Avoid repeated freezing and thawing of starting materials.
- All centrifugation steps are performed at room temperature.
- Add the following volume of 96–99.8% ethanol to each buffer bottle, close firmly, mix thoroughly and store at room temperature

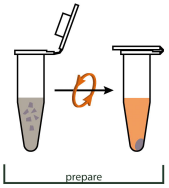
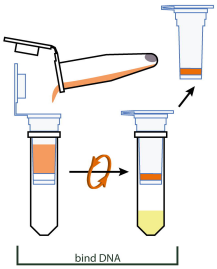
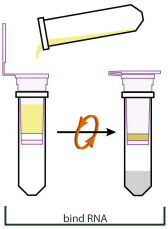
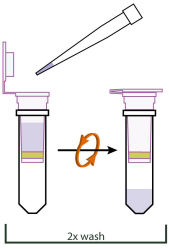
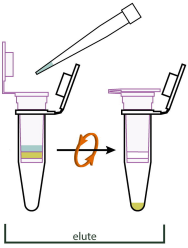
CAT. NO.		CONCENTRATE	ETHANOL	FINAL VOLUME
Buffer WASH A	BR0701901	5 ml	5 ml	10 ml
	BR0701902	15 ml	15 ml	30 ml
	BR0701903	70 ml	70 ml	140 ml
Buffer WASH B	BR0701901	6 ml	24 ml	30 ml
	BR0701902	16 ml	64 ml	80 ml
	BR0701903	36 ml	144 ml	180 ml

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
• Homogenize and lyse the sample material. • Centrifuge to pellet unlysed material.	
• Transfer the lysate to the Mini Filter DNA (blue). • Centrifuge, and discard the Mini Filter DNA with bound DNA. • The filtrate contains the RNA, don't discard!	
• Add isopropanol to the filtrate. • Transfer to a Mini Filter RNA (violet) to bind RNA and centrifuge.	
• Add Buffer WASH A and centrifuge. • Add Buffer WASH B and centrifuge. • Centrifuge once more to remove residual ethanol.	
• Add Water, RNase-free (for ELUTION), incubate and centrifuge. • microRNA in the Elution Tube is ready for use.	

PROTOCOL FOR MICRO RNA ISOLATION FROM TISSUE SAMPLES

PROCEDURE	NOTES
HOMOGENIZATION WITH A ROTOR-STATOR HOMOGENIZER • Transfer fresh or frozen starting material to a suitable reaction vessel for the homogenizer. • Add 450 µl Buffer LYSIS LR and homogenize the sample. • Transfer the homogenized tissue sample to a 1.5 ml reaction tube. HOMOGENIZATION WITH A MORTAR, PESTLE AND LIQUID NITROGEN • Transfer fresh or frozen starting material to a mortar containing liquid nitrogen and grind to a fine powder. • Transfer the powder into a 1.5 ml reaction tube. Do not allow the sample to thaw. • Add 450 µl Buffer LYSIS LR and incubate the sample at room temperature under continuous shaking until it is lysed completely (lysate becomes clear).	• Use up to 20 mg tissue. • Incomplete homogenization can reduce RNA yield. • Use the sample immediately or store at –20°C in Buffer LYSIS LR before further processing.
• Centrifuge at maximum speed for 1 minute to pellet unlysed material.	
• Transfer the lysate supernatant to a Mini Filter DNA (blue) in a Collection Tube. • Discard the reaction tube. • Centrifuge at 10,000 × *g* (~12,000 rpm) for 2 minutes. • Discard the Mini Filter DNA and keep the filtrate.	• If the solution has not completely passed through the Mini Filter DNA, centrifuge again at higher speed or prolong the centrifugation time. • Do not discard the Collection Tube containing the RNA.
• Add an equal volume isopropanol (approximately 400 µl) to the filtrate and mix the sample by pipetting up and down several times.	
• Transfer the sample to the Mini Filter RNA (violet) placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (~12,000 rpm) for 2 minutes. • Discard the Collection Tube with the filtrate.	• If the solution has not completely passed through the Mini Filter RNA, centrifuge again at higher speed or prolong the centrifugation time.
• 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 minute. • 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 minute. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH B as described above.

- Place the Mini Filter RNA in a new Collection Tube.
- Centrifuge at 10,000 × *g* (~12,000 rpm) for 3 minutes to remove all traces of ethanol.
- Discard the Collection Tube.

- Place the Mini Filter RNA into an Elution Tube.
- Add 30–80 µl Water, RNase-free (for ELUTION), to the center of the Mini Filter RNA.
- Incubate at room temperature for 1 minute.
- Centrifuge at 6,000 × *g* (~8,000 rpm) for 1 minute.
- Discard the Mini Filter RNA.

- To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION). The minimum elution volume should exceed 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).

PROTOCOL FOR MICRO RNA ISOLATION FROM EUKARYOTIC CELLS

PROCEDURE	NOTES
• Transfer up to max 5×10^6 cells to an appropriate reaction tube and pellet by centrifugation. • Discard the supernatant. • Resuspend the cells in 400 µl Buffer LYSIS LR. • Incubate at room temperature for 2 min. • Resuspend by carefully pipetting up and down, and incubate at room temperature for an additional 3 min.	• Incomplete disruption can reduce RNA yield. • No cell clumps should be visible after lysis.
• Transfer the lysate to a Mini Filter DNA (blue) in a Collection Tube. • Discard the reaction tube. • Centrifuge at 10,000 × *g* (~12,000 rpm) for 2 minutes. • Discard the Mini Filter DNA and keep the filtrate.	• If the solution has not completely passed through the Mini Filter DNA, centrifuge again at higher speed or prolong the centrifugation time. • Do not discard the Collection Tube containing the RNA.
• Add an equal volume isopropanol (approximately 400 µl) to the filtrate and mix the sample by pipetting up and down several times.	
• Transfer the sample to the Mini Filter RNA (violet) placed a new Collection Tube. • Centrifuge at 10,000 × *g* (~12,000 rpm) for 2 minutes. • Discard the Collection Tube with the filtrate.	• If the solution has not completely passed through the Mini Filter RNA, centrifuge again at higher speed or prolong the centrifugation time.
• 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 minute. • 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 minute. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH B as described above.
• Place the Mini Filter RNA into a new Collection Tube. • Centrifuge at 10,000 × *g* (~12,000 rpm) for 3 minutes to remove all traces of ethanol. • Discard the Collection Tube.	
• Place the Mini Filter into an Elution Tube. • Add 30–80 µl Water, RNase-free (for ELUTION), to the center of the Mini Filter RNA. • Incubate at room temperature for 1 min. • Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min. • Discard the Mini Filter RNA.	• To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION). Elute with at least 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).

PROTOCOL FOR MICRO RNA ISOLATION FROM BACTERIAL CELLS

PROCEDURE	NOTES
• Pellet up to 1×10^9 bacterial cells by centrifugation at 5000 × *g* (6000 rpm) for 2–5 min. • Completely remove the supernatant completely, removing drops with a pipette if necessary. • Resuspend the cell pellet completely in 100 µl TE buffer by pipetting up and down. Avoid foaming. • Add 5–10 µl (Gram-positive) or 1–2 µl (Gram-negative) 50 mg/ml lysozyme to the cell suspension. • Pipette carefully up and down until the solution becomes clear.	• Incomplete homogenization can reduce RNA yield. • Before use, prepare the lysozyme and TE buffer as described above. • The optimal amount of lysozyme and incubation time varies depending on cell type.
• Add 450 µl Buffer LYSIS LR to the clarified sample. • Resuspend by carefully pipetting up and down. • Incubate at room temperature for an additional 3 min.	• Incomplete lysis can reduce RNA yield. After lysis, lysate should be clear or viscous, with no cell clumps.
• Transfer the lysate to a Mini Filter DNA (blue) placed in a Collection Tube. • Discard the reaction tube. • Centrifuge at 10,000 × *g* (~12,000 rpm) for 2 min. • Discard the Mini Filter DNA and keep the filtrate.	• If the solution has not completely passed through the Mini Filter DNA, centrifuge again at higher speed or prolong the centrifugation time. • Do not discard the Collection Tube containing the RNA.
• Add an equal volume of isopropanol (approximately 600 µl) to the filtrate and mix by pipetting up and down.	
• Transfer 650 µl of the lysate mixture to a Mini Filter RNA (violet) placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (~12,000 rpm) for 1 minute. • Discard the Collection Tube with the filtrate.	
• Transfer the rest of the lysate mixture to the Mini Filter RNA placed in a new Collection Tube. • Centrifuge at 10,000 × *g* (~12,000 rpm) for 1 minute. • 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 minute. • 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 minute. • Discard the Collection Tube with the filtrate.	• Before use, prepare Buffer WASH B as described above.

- Place the Mini Filter RNA into a new Collection Tube.
- Centrifuge at 10,000 × *g* (~12,000 rpm) for 3 minutes to remove all traces of ethanol.
- Discard the Collection Tube.

- Place the Mini Filter into an Elution Tube.
- Add 30–80 µl Water, RNase-free (for ELUTION), to the center of the Mini Filter RNA.
- Incubate at room temperature for 1 min.
- Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min.
- Discard the Mini Filter RNA.

- To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION). Elute with at least 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 amount of starting material. After lysis, centrifuge the lysate to pellet debris and continue with the protocol using the supernatant.
LOW YIELD	
Insufficient disruption or homogenization	Reduce amount of starting material. Avoid overloading the Mini Filter, as overloading reduces yield.
Incomplete elution	To improve elution, prolong the incubation time to 5 min or repeat elution.
DNA CONTAMINATION	
Too much starting material	Reduce amount of starting material.
Incorrect lysis of starting material	Use the recommended techniques for lysis. Perform an on-column DNase digestion step after binding the RNA to the Mini Filter RNA (violet). Alternatively, perform DNase digestion of the eluate. Ensure the DNase I is RNase-free.
DEGRADED RNA	
Starting material inappropriately handled or stored	Make sure that the starting material is fresh. Perform the protocol, especially the first steps, quickly.
RNase contamination	Use sterile, RNase-free filter tips. Before every preparation clean the pipette, the devices and the working place. Always wear gloves.
RNA DOES NOT PERFORM WELL IN DOWNSTREAM APPLICATIONS (E.G., RT-PCR)	
Contamination of eluate with ethanol	Increase centrifuge time for removing ethanol.
Contamination of eluate with salt	Ensure that Buffer WASH A and Buffer WASH B are at room temperature before use. Check the washing solutions for salt precipitates. Dissolve any precipitate 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!

CERTIFICATE OF ANALYSIS

The components of the kit were tested for isolation of total RNA and enrichment of small RNA molecules by 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

Legal Disclaimer and Product Use Limitation

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