GenUP™ BS Virus RNA Kit

LOT: See product label EXPIRY DATE: See product label

ORDERING INFORMATION

PRODUCT	GenUP™ BS Virus RNA Kit
CAT. NO.	BR0702303
SIZE	250 preps
COMPONENTS	
Buffer LYSIS LD	150 ml
CARRIER RNA (lyophilized)	3 vials (add 1 ml Water, RNase-free)
Proteinase K (lyophilized)	2 vials (add 1.5 ml double-distilled water)
Buffer WASH B (concentrate)	36 ml (add 144 ml ethanol, absolute)
Water, RNase-free	30 ml
Mini Filters	250
Collection Tubes (2 ml)	250

STORAGE

Room temperature (until expiry date – see product label).
If precipitation appears, gently warm the solution to dissolve the precipitate.
Store lyophilized Proteinase K and CARRIER RNA at 4°C,
Store aliquots of dissolved Proteinase K and CARRIER RNA at −20°C, avoid repeated freezing and thawing.

FEATURES

- Fast and simple procedure
- High yields of pure RNA
- No DNase treatment, no toxic β-mercaptoethanol

APPLICATIONS

- Virus RNA isolation from swabs

DESCRIPTION

biotechrabbit™ GenUP BS Virus RNA Kit has been specially developed for quick and easy isolation of viral RNA from swabs, including nasopharyngeal and oropharyngeal swabs. The unique binding membrane of our high-capacity Mini Filters guaranties high yields. A high concentration of purified RNA can be achieved with flexible elution volumes. The kit includes carrier RNA.
After few initial steps, the viral RNA is bound to a Mini Filter, washed, and then eluted in a separate tube. The purified RNA is ready to be used in all demanding molecular biology applications, including qRT-PCR.

SPECIFICATIONS

STARTING MATERIAL	Swab samples
EXTRACTION TIME	Approximately 25 min
TYPICAL YIELD	Yield is dependent on sample quality and viral load

MATERIALS SUPPLIED BY THE USER

- Absolute ethanol (molecular biology grade, undenaturated)
- 80% ethanol (molecular biology grade, undenaturated)
- 2-Propanol (molecular biology grade)
- Double-distilled water for dissolving Proteinase K
- 1.5 ml and 2 ml tubes
- Pipet tips

STEPS BEFORE STARTING

Initial steps

- Add 144 ml absolute ethanol (molecular biology grade, undenaturated) to Buffer WASH B
- Add 1.5 ml double-distilled water to each vial Proteinase K, mix thoroughly and store aliquots at −20°C.
- Add 1 ml Water, RNase-free (in the kit) to each vial CARRIER RNA, mix thoroughly and store aliquots at −20°C. CARRIER contains RNA, do not contaminate with RNases.
- Avoid repeated freezing and thawing.
- Pre-heat water bath or thermal mixer to 70°C.
- Calculate and transfer the amount of Water, RNase-free (in the kit) needed for elution into a 1.5 ml reaction tube and pre-heat to 70°C.
- Ensure that Buffer WASH B is at room temperature. Dissolve any salt precipitates by carefully warming.

In order to monitor the extraction and subsequent detection, the use of internal standards as well as positive and negative controls are recommended. In case CARRIER RNA is inhibiting a PCR reaction, the amount of CARRIER RNA used during extraction may have to be optimized.

- If the extraction tubes used are coated with carrier nucleic acids and internal control RNA, it is not necessary to use the CARRIER RNA. The minimum amount of CARRIER RNA should be optimized for each PCR method. Excessive CARRIER RNA can inhibit PCR.
- The use of an internal control RNA as well as positive and negative controls to monitor the purification, amplification and detection processes is highly recommended. Control RNA can be added after lysis and before applying the sample on the column.
- Perform all centrifugation steps at room temperature.
- Before elution, the necessary volume of Water, RNase-free (for ELUTION), must be warmed to 70°C.
- Final eluates contain both viral RNA and CARRIER RNA, and therefore, the photometric or fluorometric quantification of nucleic acids is not relevant. qPCR is recommended to quantify the purified RNA.
- The sensitivity of virus detection is highly dependent on the procedure used (standard PCR or commercial detection kits).
- Mark all vials and filters to avoid confusion when purifying multiple samples.

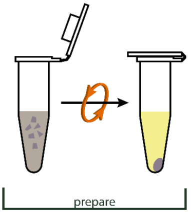
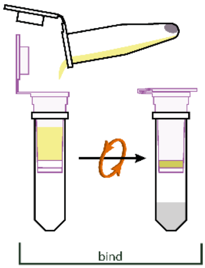
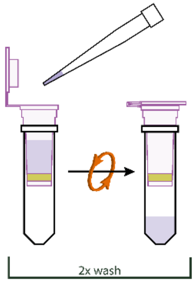
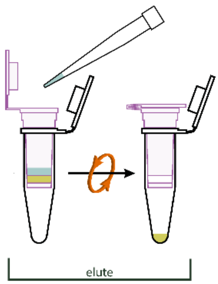
GUIDELINES FOR PREVENTION OF RNA DEGRADATION

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
• Extract swab and lyse with Proteinase K. • Centrifuge.	
• Add isopropanol, mix and transfer to a Mini Filter. • Centrifuge.	
• Add Buffer WASH B and centrifuge. • Wash 2 times with 80% ethanol and centrifuge. • Centrifuge once more to remove residual ethanol.	
• Add pre-heated Water, RNase-free, incubate and centrifuge. • RNA is ready for use.	

PROTOCOL FOR DIRECT ISOLATION OF VIRAL RNA FROM SWABS

PROCEDURE	NOTES
• Add 600 µl Buffer LYSIS LD into a 2.0 ml reaction tube. • Incubate the swab for 10 min inside the closed tube. Shake the swab vigorously and squeeze it as complete as possible to collect maximum liquid sample. Discard the swab. • Transfer 400 µl of the particle-free sample into a new 1.5 ml reaction tube. • Add 10 µl CARRIER RNA and 10 µl Proteinase K. • Mix by pulse vortexing for 10 s. • Incubate at 70°C for 10 min. • Centrifuge briefly to collect the liquid and condensate at the bottom of the tube.	• 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.
• Add 400 µl isopropanol to the lysate and mix by vortexing or by pipetting up and down several times.	Mix carefully to get a homogeneous solution. Option: Add internal control at this step.
• Apply the complete sample to a Mini Filter placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and reuse the Collection Tube.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.
• Add 650 µl Buffer WASH B to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and reuse the Collection Tube.	• Before use, prepare Buffer WASH B as described above.
• Add 650 µl 80% ethanol to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and reuse the Collection Tube.	
• Add 650 µl 80% ethanol to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 30 s. • Discard the filtrate and reuse the Collection Tube.	
• Centrifuge again for 4 min to remove residual ethanol. • Discard the Collection Tube.	Ethanol is a potent PCR inhibitor. Avoid contamination of the Mini Filter with the filtrate after centrifugation.
• Place the Mini Filter into a new 1.5 ml reaction tube. • Add 60 µl pre-heated Water, RNase-free, to the center of the Mini Filter. • Incubate at room temperature for 2 min. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Mini Filter.	• Before use, pre-heat the Water, RNase-free to 70°C. • To improve yield, perform elution twice using ½ volume of Water, RNase-free.
• 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 ISOLATION OF VIRAL RNA FROM SWABS STORED UNDER PHYSIOLOGICAL SALINE

PROCEDURE	NOTES
• Shake the storage tube with the swab stored in PBS vigorously for 10 min. Squeeze the swab as complete as possible to collect maximum liquid sample. Discard the swab. • Add 200 µl Buffer LYSIS LD and 10 µl CARRIER RNA into a new 1.5 ml reaction tube. • Add 200 µl of the particle-free sample. • Add 10 µl Proteinase K. • Mix by pulse vortexing for 10 s. • Incubate at 70°C for 10 min. • Centrifuge briefly to collect the liquid and condensate at the bottom of the tube.	• 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.
• Add 400 µl isopropanol to the lysate and mix by vortexing or by pipetting up and down several times.	Mix carefully to get a homogeneous solution. Option: Add internal control at this step.
• Apply the complete sample to a Mini Filter placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and reuse the Collection Tube.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.
• Add 650 µl Buffer WASH B to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and reuse the Collection Tube.	• Before use, prepare Buffer WASH B as described above.
• Add 650 µl 80% ethanol to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and reuse the Collection Tube.	
• Add 650 µl 80% ethanol to the Mini Filter. • Centrifuge at 10,000 × *g* (12,000 rpm) for 30 s. • Discard the filtrate and reuse the Collection Tube.	
• Centrifuge again for 4 min to remove residual ethanol. • Discard the Collection Tube.	Ethanol is a potent PCR inhibitor. Avoid contamination of the Mini Filter with the filtrate after centrifugation.
• Place the Mini Filter into a new 1.5 ml reaction tube. • Add 60 µl pre-heated Water, RNase-free, to the center of the Mini Filter. • Incubate at room temperature for 2 min. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the Mini Filter.	• Before use, pre-heat the Water, RNase-free to 70°C. • To improve yield, perform elution twice using ½ volume of Water, RNase-free.
• 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 MINI FILTER	
Excessive starting material, insufficient lysis	Increase lysis time. Remove debris by centrifugation and continue with the supernatant. Reduce the amount of starting material.
LOW YIELD	
Excessive starting material, insufficient lysis	Increase lysis time. Reduce the amount of starting material. Overloading reduces yield.
Incomplete elution	Prolong the elution time up to 5 min. Repeat elution. Use a higher volume of Water, RNase-free.
Insufficient mixing with isopropanol	Ensure the sample and isopropanol are mixed to homogeneity.
LOW RNA CONCENTRATION	
Excessive elution volume	Prewarm the Water, RNase-free as described. Do not exceed the recommended volume of water. Perform two elution steps with half of the total elution volume. The first eluate normally exhibits a higher RNA concentration than the second eluate.
No CARRIER RNA used	Use the CARRIER RNA as described to increase yield and nucleic acid concentration.
RNA DOES NOT PERFORM WELL IN OTHER APPLICATIONS (RT-PCR)	
Ethanol carryover	Increase centrifugation time for removing ethanol. Do not contaminate the Mini Filter with the filtrate after centrifugation.
Salt carryover during elution	Ensure that Buffer WASH B is at room temperature. If a buffer contains salt precipitate, dissolve the precipitate by warming carefully.

SAFETY PRECAUTIONS

- This kit is made for single use only!
- Do not 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!

CERTIFICATE OF ANALYSIS

The components of the kit were tested for viral RNA purification from swabs and subsequent analysis of purified RNA in qPCR.

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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