

## GenUP™ Virus DNA/RNA Kit

LOT: See product label

EXPIRY DATE: See product label

## ORDERING INFORMATION

| PRODUCT                         | GenUP™ Virus DNA/RNA Kit                     |  |   |           |
|---------------------------------|--|--|---|-----------|
|                                 | CAT. NO.                                     | BR0701101                                    | BR0701102                                     | BR0701103 |
| SIZE                            | 10 preps                                     | 50 preps                                     | 250 preps                                     |           |
| COMPONENTS                      |  |  |   |           |
| Buffer LYSIS LD                 | 10 ml  | 25 ml  | 125 ml  |           |
| Buffer BINDING BD               | 16 ml  | 60 ml  | 230 ml  |           |
| CARRIER (lyophilized)           | 1 vial<br>(add 1.25 ml Water,<br>RNase-free) | 1 vial<br>(add 1.25 ml Water,<br>RNase-free) | 3 vials<br>(add 1.25 ml Water,<br>RNase-free) |           |
| Water, RNase-free (for CARRIER) | 2 ml   | 2 ml   | 3 × 2 ml                                      |           |
| Proteinase K (lyophilized)      | 1 vial<br>(add 0.3 ml water)                 | 1 vial<br>(add 1.5 ml water)                 | 4 vials<br>(add 1.5 ml water)                 |           |
| Buffer WASH A (concentrate)     | 5 ml<br>(add 5 ml ethanol)                   | 15 ml<br>(add 15 ml ethanol)                 | 70 ml<br>(add 70 ml ethanol)                  |           |
| Buffer WASH B (concentrate)     | 6 ml<br>(add 24 ml ethanol)                  | 16 ml<br>(add 64 ml ethanol)                 | 2 × 36 ml<br>(add 144 ml ethanol)             |           |
| Water, RNase-free (for ELUTION) | 2 ml   | 2 × 2 ml                                     | 25 ml   |           |
| Mini Filters (blue)             | 10   | 50   | 5 × 50  |           |
| Collection Tubes (2 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 lyophilized Proteinase K and CARRIER at 4°C,

Store aliquots of dissolved Proteinase K and CARRIER at -20°C.

Store lyophilized and dissolved Carrier RNA at -22 °C to -18 °C.

# GenUP™ Virus DNA/RNA Kit

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## FEATURES

- Universal kit for simultaneous RNA and DNA isolation from different starting materials
- Fast and simple procedure with sample-specific protocols
- High yields of pure RNA and DNA
- No DNase treatment, no toxic  $\beta$ -mercaptoethanol

## APPLICATIONS

- Simultaneous viral DNA and RNA isolation from various sources
- Excellent performance for unknown viruses

## DESCRIPTION

biotechrabbit™ GenUP Virus DNA/RNA Kit has been specially developed for quick and easy isolation of viral RNA and DNA. The kit is especially useful when the origin of the virus is unknown. Viral double-stranded DNA and single-stranded RNA are simultaneously isolated from eukaryotic samples, including plasma, serum, and other body fluids as well as cell cultures, tissues, and buccal swabs.

The unique binding membrane of our high-capacity Mini Filters guarantees high yields. A high concentration of purified nucleic acid can be achieved with flexible elution volumes. The kit includes carrier RNA.

After a few initial procedures, the viral nucleic acids are bound to a Mini Filter, washed and then eluted in a separate tube. The purified nucleic acids are ready to be used in all demanding molecular biology applications, including cDNA synthesis, northern blot analysis, qPCR and RT-PCR.

## SPECIFICATIONS

|                          |   |
|--------------------------|---|
| <b>STARTING MATERIAL</b> | Eukaryotic cells (up to $5 \times 10^6$ )<br>Serum, plasma, cell-free body fluids, cell culture supernatants (150 $\mu$ l)<br>Tissue samples, biopsies (up to 20 mg)<br>Paraffin-embedded tissues, buccal swabs |
|--------------------------|---|

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|                        |                      |
|------------------------|----------------------|
| <b>EXTRACTION TIME</b> | Approximately 25 min |
|------------------------|----------------------|

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|                      |  |
|----------------------|--|
| <b>TYPICAL YIELD</b> | Yield is highly dependent on sample type |
|----------------------|--|

## MATERIALS SUPPLIED BY THE USER

- 96–99.8% ethanol
- Double-distilled water, RNase-free
- PBS (Phosphate buffered saline; 137 mM NaCl, 2.7 mM KCl, 10 mM  $\text{Na}_2\text{HPO}_4$ , 1.8 mM  $\text{KH}_2\text{PO}_4$ ; pH 7.4) for tissue and biopsy samples
- 0.9% NaCl for swabs
- Centrifugation tubes
- Pipet tips

## STEPS BEFORE STARTING

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

| CAT. NO.      | CONCENTRATE | ETHANOL | FINAL VOLUME |
|---------------|-------------|---------|--------------|
| Buffer WASH A | BR0701101   | 5 ml    | 5 ml         |
|               | BR0701102   | 15 ml   | 15 ml        |
|               | BR0701103   | 70 ml   | 70 ml        |
| Buffer WASH B | BR0701101   | 6 ml    | 24 ml        |
|               | BR0701102   | 16 ml   | 64 ml        |
|               | BR0701103   | 36 ml   | 144 ml       |

- Add the following volume of double-distilled water, RNase-free to each vial Proteinase K, mix thoroughly and store 0.3 ml aliquots at  $-20^{\circ}\text{C}$ . Avoid repeated freezing and thawing.

BR0701101                      0.3 ml

BR0701102, BR0701103      1.5 ml for 5 × 0.3 ml aliquots

- Add 1.25 ml Water, RNase-free (for CARRIER), to each vial CARRIER, mix thoroughly and store 0.25 ml aliquots at  $-20^{\circ}\text{C}$ . CARRIER contains RNA: avoid contamination with RNases.
- Before the each purification, prepare a fresh mix of CARRIER and Buffer LYSIS LD. Store at  $4^{\circ}\text{C}$  and use within one day.
- Mixing scheme for protocols using 200  $\mu\text{l}$  CARRIER-Buffer LYSIS LD aliquots.

| COMPONENT                          | FOR 5 SAMPLES    | FOR 10 SAMPLES    | FOR N SAMPLES         |
|------------------------------------|------------------|-------------------|-----------------------|
| Buffer LYSIS LD                    | 1.2 ml           | 2.4 ml            | 240 $\mu\text{l}$ × n |
| CARRIER solution prepared as above | 60 $\mu\text{l}$ | 120 $\mu\text{l}$ | 12 $\mu\text{l}$ × n  |
| Final volume                       | 1.26 ml          | 2.52 ml           | 252 $\mu\text{l}$ × n |

- Mixing scheme for protocols using 400  $\mu\text{l}$  CARRIER-Buffer LYSIS LD aliquots.

| COMPONENT                          | FOR 5 SAMPLES    | FOR 10 SAMPLES    | FOR N SAMPLES         |
|------------------------------------|------------------|-------------------|-----------------------|
| Buffer LYSIS LD                    | 2.4 ml           | 4.8 ml            | 480 $\mu\text{l}$ × n |
| CARRIER solution prepared as above | 60 $\mu\text{l}$ | 120 $\mu\text{l}$ | 12 $\mu\text{l}$ × n  |
| Final volume                       | 2.46 ml          | 4.92 ml           | 492 $\mu\text{l}$ × n |

- If the extraction tubes used are coated with carrier nucleic acids and internal control RNA, it is not necessary to use the CARRIER. The minimum amount of CARRIER should be optimized for each PCR method. Excessive CARRIER 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 when preparing the CARRIER–Buffer LYSIS LD mix.
- 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, 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.

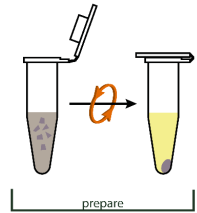
- Always wear gloves, and change them frequently.
- Keep all tubes closed when possible.
- Keep samples and isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free).
- Non-disposable 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

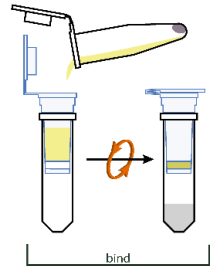
## STEPS

- Homogenize and lyse the sample material.
- Centrifuge to pellet debris.

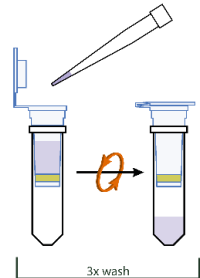
## SCHEME



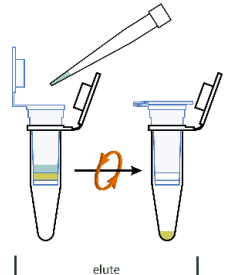
- Add Buffer BINDING BD and transfer to a Mini Filter (blue).
- Centrifuge.



- 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.
- DNA/RNA in the Elution Tube is ready for use.



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## PROTOCOL FOR ISOLATION OF VIRAL DNA/RNA FROM SERUM, PLASMA, CELL-FREE BODY FLUIDS AND CELL CULTURE SUPERNATANTS UP TO 200 µL

| PROCEDURE   | NOTES  |
|---|--|
| <ul style="list-style-type: none"><li>• Transfer 200 µl fresh CARRIER–Buffer LYSIS LD mix to an empty 1.5 ml reaction tube.</li><li>• Add 200 µl sample (serum, plasma, cell-free body fluids or cell culture supernatants).</li><li>• Add 20 µl Proteinase K.</li><li>• Mix by pulse vortexing for 10 s.</li><li>• Incubate at 37–70°C until the sample is completely lysed (approximately 10–30 min).</li><li>• Centrifuge briefly to collect the liquid at the bottom of the tube.</li></ul> | <ul style="list-style-type: none"><li>• Before use, prepare the CARRIER–Buffer LYSIS LD mix as described above.</li><li>• 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.</li><li>• The solution becomes clear when the lysis is complete. Incubation time depends on sample and temperature. Incomplete lysis can decrease RNA yield.</li></ul> |
| <ul style="list-style-type: none"><li>• Add 400 µl Buffer BINDING BD to the lysate, and mix by vortexing or by pipetting up and down several times.</li></ul>   | <p>Option: Add internal control at this step.</p>  |
| <ul style="list-style-type: none"><li>• Apply the sample to a Mini Filter (blue) placed in a Collection Tube.</li><li>• Centrifuge at 10,000 × g (12,000 rpm) for 1 min.</li><li>• Discard the Collection Tube with the filtrate.</li></ul>   | <ul style="list-style-type: none"><li>• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.</li></ul>  |
| <ul style="list-style-type: none"><li>• Add 500 µl Buffer WASH A to the Mini Filter placed in a new Collection Tube.</li><li>• Centrifuge at 10,000 × g (12,000 rpm) for 1 min.</li><li>• Discard the Collection Tube with the filtrate.</li></ul>  | <ul style="list-style-type: none"><li>• Before use, prepare Buffer WASH A as described above.</li></ul>  |
| <ul style="list-style-type: none"><li>• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube.</li><li>• Centrifuge at 10,000 × g (12,000 rpm) for 1 min.</li><li>• Discard the Collection Tube with the filtrate.</li></ul>  | <ul style="list-style-type: none"><li>• Before use, prepare Buffer WASH B as described above.</li></ul>  |
| <ul style="list-style-type: none"><li>• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube.</li><li>• Centrifuge at 10,000 × g (12,000 rpm) for 1 min.</li><li>• Discard the Collection Tube with the filtrate.</li></ul>  |  |
| <ul style="list-style-type: none"><li>• Place the Mini Filter into a new Collection Tube.</li><li>• Centrifugation again for 5 min to remove residual ethanol.</li><li>• Discard the Collection Tube.</li></ul>   |  |
| <ul style="list-style-type: none"><li>• Place the Mini Filter into an Elution Tube.</li><li>• Add 60 µl warm Water, RNase-free (for ELUTION), to the center of the Mini Filter.</li><li>• Incubate at room temperature for 2 min.</li><li>• Centrifuge at 8,000 × g (10,000 rpm) for 1 min.</li><li>• Discard the Mini Filter.</li></ul>  | <ul style="list-style-type: none"><li>• Before use, prewarm the Water, RNase-free (for ELUTION), to 70°C.</li><li>• To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION).</li></ul>   |
| <ul style="list-style-type: none"><li>• Purified DNA/RNA in the Elution Tube can be used immediately.</li></ul>   | <ul style="list-style-type: none"><li>• Store the DNA/RNA at 4°C (short-term) or –80°C (long-term).</li></ul>  |

## PROTOCOL FOR ISOLATION OF VIRAL DNA/RNA FROM SERUM, PLASMA, CELL-FREE BODY FLUIDS AND CELL CULTURE SUPERNATANTS UP TO 400 µL

| PROCEDURE  | NOTES  |
|--|--|
| <ul style="list-style-type: none"> <li>• Transfer 400 µl fresh CARRIER–Buffer LYSIS LD mix to an empty 2 ml reaction tube.</li> <li>• Add 400 µl sample (serum, plasma, cell-free body fluids or cell culture supernatants).</li> <li>• Add 20 µl Proteinase K.</li> <li>• Mix by pulse vortexing for 10 s.</li> <li>• Incubate at 37–70°C until the sample is completely lysed (approximately 10–30 min).</li> <li>• Centrifuge briefly to collect the liquid at the bottom of the tube.</li> </ul> | <ul style="list-style-type: none"> <li>• Before use, prepare the CARRIER–Buffer LYSIS LD mix as described above.</li> <li>• 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.</li> <li>• The solution becomes clear when the lysis is complete. Incubation time depends on sample and temperature. Incomplete lysis can decrease RNA yield.</li> </ul> |
| <ul style="list-style-type: none"> <li>• Add 800 µl Buffer BINDING BD to the lysate, and mix by vortexing or by pipetting up and down several times.</li> </ul>  | <p>Option: Add internal control at this step.</p>  |
| <ul style="list-style-type: none"> <li>• Apply 650 µl sample to a Mini Filter (blue) placed in a Collection Tube.</li> <li>• Centrifuge at <math>10,000 \times g</math> (12,000 rpm) for 1 min.</li> <li>• Discard the Collection Tube with the filtrate.</li> </ul>   | <ul style="list-style-type: none"> <li>• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.</li> </ul>  |
| <ul style="list-style-type: none"> <li>• Apply the remaining sample volume to the Mini Filter placed in a new Collection Tube.</li> <li>• Centrifuge at <math>10,000 \times g</math> (12,000 rpm) for 1 min.</li> <li>• Discard the Collection Tube with the filtrate.</li> </ul>  |  |
| <ul style="list-style-type: none"> <li>• Add 500 µl Buffer WASH A to the Mini Filter placed in a new Collection Tube.</li> <li>• Centrifuge at <math>10,000 \times g</math> (12,000 rpm) for 1 min.</li> <li>• Discard the Collection Tube with the filtrate.</li> </ul>   | <ul style="list-style-type: none"> <li>• Before use, prepare Buffer WASH A as described above.</li> </ul>  |
| <ul style="list-style-type: none"> <li>• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube.</li> <li>• Centrifuge at <math>10,000 \times g</math> (12,000 rpm) for 1 min.</li> <li>• Discard the Collection Tube with the filtrate.</li> </ul>   | <ul style="list-style-type: none"> <li>• Before use, prepare Buffer WASH B as described above.</li> </ul>  |
| <ul style="list-style-type: none"> <li>• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube.</li> <li>• Centrifuge at <math>10,000 \times g</math> (12,000 rpm) for 1 min.</li> <li>• Discard the Collection Tube with the filtrate.</li> </ul>   |  |
| <ul style="list-style-type: none"> <li>• Place the Mini Filter into a new Collection Tube.</li> <li>• Centrifugation again for 5 min to remove residual ethanol.</li> <li>• Discard the Collection Tube.</li> </ul>  |  |

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- Place the Mini Filter into an Elution Tube.
  - Add 60 µl warm Water, RNase-free (for ELUTION), to the center of the Mini Filter.
  - Incubate at room temperature for 2 min.
  - Centrifuge at 8,000 × g (10,000 rpm) for 1 min.
  - Discard the Mini Filter.
- 
- Before use, prewarm the Water, RNase-free (for ELUTION), to 70°C.
  - To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION).
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- Purified DNA/RNA in the Elution Tube can be used immediately.
  - Store the DNA/RNA at 4°C (short-term) or –80°C (long-term).

## PROTOCOL FOR ISOLATION OF VIRAL DNA/RNA FROM TISSUE SAMPLES AND BIOPSIES

### PROCEDURE

### NOTES

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- Cut up to 20 mg tissue sample or biopsy into small pieces.
  - Add 9 volumes PBS buffer or Water, RNase-free, to make a 10% (w/v) tissue suspension.
  - Homogenized the sample using a commercial homogenization tool (bead-based or other).
- 
- Centrifuge at maximum speed for 2 min to pellet debris. Use the supernatant in the next step.
- 
- Transfer 200 µl fresh CARRIER–Buffer LYSIS LD mix to an empty 1.5 ml reaction tube.
  - Add 200 µl particle-free sample (homogenized tissue).
  - Add 20 µl Proteinase K.
  - Mix by pulse vortexing for 10 s.
  - Incubate at 37–70°C until the sample is completely lysed (approximately 10–30 min).
  - Centrifuge briefly to collect the liquid at the bottom of the tube.
- 
- Add 400 µl Buffer BINDING BD to the lysate, and mix by vortexing or by pipetting up and down several times.
- 
- Apply the sample to a Mini Filter (blue) placed in a Collection Tube.
  - Centrifuge at 10,000 × g (12,000 rpm) for 1 min.
  - Discard the Collection Tube with the filtrate.
- 
- Insufficient homogenization can decrease DNA/RNA yields.
  - Avoid repeated freezing and thawing of tissue samples.
- 
- Before use, prepare the CARRIER–Buffer LYSIS LD mix 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.
  - The solution becomes clear when the lysis is complete. Incubation time depends on sample and temperature. Incomplete lysis can decrease RNA yield.
- 
- Option: Add internal control at this step.
- 
- If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.



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| <ul style="list-style-type: none"> <li>• Add 500 µl Buffer WASH A to the Mini Filter placed in a new Collection Tube.</li> <li>• Centrifuge at 10,000 × g (12,000 rpm) for 1 min.</li> <li>• Discard the Collection Tube with the filtrate.</li> </ul> | <ul style="list-style-type: none"> <li>• Before use, prepare Buffer WASH A as described above.</li> </ul> |
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| <ul style="list-style-type: none"> <li>• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube.</li> <li>• Centrifuge at 10,000 × g (12,000 rpm) for 1 min.</li> <li>• Discard the Collection Tube with the filtrate.</li> </ul> | <ul style="list-style-type: none"> <li>• Before use, prepare Buffer WASH B as described above.</li> </ul> |
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| <ul style="list-style-type: none"> <li>• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube.</li> <li>• Centrifuge at 10,000 × g (12,000 rpm) for 1 min.</li> <li>• Discard the Collection Tube with the filtrate.</li> </ul> |  |
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| <ul style="list-style-type: none"> <li>• Place the Mini Filter into a new Collection Tube.</li> <li>• Centrifugation again for 5 min to remove residual ethanol.</li> <li>• Discard the Collection Tube.</li> </ul> |  |
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| <ul style="list-style-type: none"> <li>• Place the Mini Filter into an Elution Tube.</li> <li>• Add 60 µl warm Water, RNase-free (for ELUTION), to the center of the Mini Filter.</li> <li>• Incubate at room temperature for 2 min.</li> <li>• Centrifuge at 8,000 × g (10,000 rpm) for 1 min.</li> <li>• Discard the Mini Filter.</li> </ul> | <ul style="list-style-type: none"> <li>• Before use, prewarm the Water, RNase-free (for ELUTION), to 70°C.</li> <li>• To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION).</li> </ul> |
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| <ul style="list-style-type: none"> <li>• Purified DNA/RNA in the Elution Tube can be used immediately.</li> </ul> | <ul style="list-style-type: none"> <li>• Store the DNA/RNA at 4°C (short-term) or –80°C (long-term).</li> </ul> |
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## PROTOCOL FOR ISOLATION OF VIRAL DNA/RNA FROM SWABS

### PROCEDURE

- Transfer 0.9% NaCl to a 1.5 ml reaction tube.
  - Submerge the swab in the liquid. If the shaft of the swab interferes with closing the tube, it can be cut off.
  - Incubate at room temperature for 15 min.
- 
- After incubation, mix well. Shake the liquid from the swab into the tube, and squeeze the swab on the wall of the tube to transfer as much liquid as possible to the tube.
  - Discard the swab.

### NOTES

- Before use, prepare the 0.9% NaCl as described above.
- Insufficient lysis can decrease DNA/RNA yield.

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| <ul style="list-style-type: none"><li>• Transfer 200 µl fresh CARRIER–Buffer LYSIS LD mix to an empty 1.5 ml reaction tube.</li><li>• Add 200 µl particle-free sample (liquid from swab).</li><li>• Add 20 µl Proteinase K.</li><li>• Mix by pulse vortexing for 10 s.</li><li>• Incubate at 37–70°C until the sample is completely lysed (approximately 10–30 min).</li><li>• Centrifuge briefly to collect the liquid at the bottom of the tube.</li></ul> | <ul style="list-style-type: none"><li>• Before use, prepare the CARRIER–Buffer LYSIS LD mix as described above.</li><li>• 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.</li><li>• The solution becomes clear when the lysis is complete. Incubation time depends on sample and temperature. Incomplete lysis can decrease RNA yield.</li></ul> |
| <ul style="list-style-type: none"><li>• Add 400 µl Buffer BINDING BD to the lysate, and mix by vortexing or by pipetting up and down several times.</li></ul>  | <p>Option: Add internal control at this step.</p>  |
| <ul style="list-style-type: none"><li>• Apply the sample to a Mini Filter (blue) placed in a Collection Tube.</li><li>• Centrifuge at 10,000 × <i>g</i> (12,000 rpm) for 1 min.</li><li>• Discard the Collection Tube with the filtrate.</li></ul>   | <ul style="list-style-type: none"><li>• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.</li></ul>  |
| <ul style="list-style-type: none"><li>• Add 500 µl Buffer WASH A to the Mini Filter placed in a new Collection Tube.</li><li>• Centrifuge at 10,000 × <i>g</i> (12,000 rpm) for 1 min.</li><li>• Discard the Collection Tube with the filtrate.</li></ul>  | <ul style="list-style-type: none"><li>• Before use, prepare Buffer WASH A as described above.</li></ul>  |
| <ul style="list-style-type: none"><li>• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube.</li><li>• Centrifuge at 10,000 × <i>g</i> (12,000 rpm) for 1 min.</li><li>• Discard the Collection Tube with the filtrate.</li></ul>  | <ul style="list-style-type: none"><li>• Before use, prepare Buffer WASH B as described above.</li></ul>  |
| <ul style="list-style-type: none"><li>• Add 650 µl Buffer WASH B to the Mini Filter placed in a new Collection Tube.</li><li>• Centrifuge at 10,000 × <i>g</i> (12,000 rpm) for 1 min.</li><li>• Discard the Collection Tube with the filtrate.</li></ul>  |  |
| <ul style="list-style-type: none"><li>• Place the Mini Filter into a new Collection Tube.</li><li>• Centrifugation again for 5 min to remove residual ethanol.</li><li>• Discard the Collection Tube.</li></ul>  |  |
| <ul style="list-style-type: none"><li>• Place the Mini Filter into an Elution Tube.</li><li>• Add 60 µl warm Water, RNase-free (for ELUTION), to the center of the Mini Filter.</li><li>• Incubate at room temperature for 2 min.</li><li>• Centrifuge at 8,000 × <i>g</i> (10,000 rpm) for 1 min.</li><li>• Discard the Mini Filter.</li></ul>  | <ul style="list-style-type: none"><li>• Before use, prewarm the Water, RNase-free (for ELUTION), to 70°C.</li><li>• To improve yield, perform elution twice using ½ volume of Water, RNase-free (for ELUTION).</li></ul>   |
| <ul style="list-style-type: none"><li>• Purified DNA/RNA in the Elution Tube can be used immediately.</li></ul>  | <ul style="list-style-type: none"><li>• Store the DNA/RNA at 4°C (short-term) or –80°C (long-term).</li></ul>  |

## TROUBLESHOOTING

| PROBLEM  | SOLUTION  |
|--|---|
| <b>CLOGGED MINI FILTER</b>   |   |
| Excessive starting material, insufficient lysis                            | Follow recommendations for the maximum amount of starting material. Perform lysis until the solution becomes clear and viscosity decreases.   |
| <b>LOW YIELD</b>   |   |
| Excessive starting material, insufficient lysis                            | Follow recommendations for the maximum amount of starting material. Perform lysis until the solution becomes clear and viscosity decreases. Higher temperatures are more effective.   |
| Incomplete elution   | Prolong the elution incubation up to 5 min. Repeat elution. Use the recommended volume of Water, RNase-free (for ELUTION).  |
| Incorrect binding  | Ensure the sample and Buffer BINDING BD are mixed to homogeneity.   |
| <b>LOW DNA/RNA CONCENTRATION</b>   |   |
| Excessive elution volume   | Prewarm the Water, RNase-free (for ELUTION), as described. Do not exceed the recommended volume of water. Perform two elution steps each with half of the total elution volume. The first eluate normally exhibits a higher RNA concentration than the second eluate. |
| No CARRIER used  | Use the CARRIER as described to increase yield and nucleic acid concentration.  |
| <b>DEGRADED RNA</b>  |   |
| 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 of solutions, tubes, etc.                              | Use sterile, RNase-free filter tips. Before every RNA preparation, clean the pipette, devices and working place. Always wear gloves.  |
| <b>DNA/RNA DOES NOT PERFORM WELL IN OTHER APPLICATIONS (PCR OR RT-PCR)</b> |   |
| Ethanol carryover  | Increase centrifugation time for removing ethanol.  |
| Salt carryover during elution  | Ensure that Buffer WASH A and Buffer WASH B are at room temperature. If a buffer contains precipitate, dissolve the 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!
- Buffer WASH A contains guanidine isothiocyanate, which is harmful to health. Contact with acids liberates very toxic gas!

# GenUP™ Virus DNA/RNA Kit

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## CERTIFICATE OF ANALYSIS

The components of the kit were tested for simultaneous viral RNA and DNA purification from liquid samples and subsequent analysis of purified nucleic acids in qPCR. The kit is tested for following viruses: hepatitis B, cytomegalovirus, Epstein–Barr virus and herpes simplex.

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](http://www.biotechrabbit.com) for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

## CONTACT BIOTECHRABBIT

biotechrabbit GmbH  
Volmerstr. 9a  
12489 Berlin, Germany

[info@biotechrabbit.com](mailto:info@biotechrabbit.com)  
[support@biotechrabbit.com](mailto:support@biotechrabbit.com)  
[www.biotechrabbit.com](http://www.biotechrabbit.com)

Phone: +49 30 555 7821-10  
Fax: +49 30 555 7821-99



### *Legal Disclaimer and Product Use Limitation*

*Purchase of product does not include a license to perform any patented applications; therefore it is the sole responsibility of users to determine whether they may be required to engage a license agreement depending upon the particular application in which the product is used. This product was developed, manufactured, and sold for in vitro use only. It is not suitable for administration to humans or animals.*

*Trademarks: biotechrabbit™, GenUP™ (biotechrabbit GmbH).*

valid from 26.07.2021