Virus-RNA Isolation-Kit

Data sheet

Order-No.	
BS77.861.0010	10 Preparations
BS77.861.0050	50 Preparations
BS77.861.0250	250 Preparations

(For research and *in vitro* use only)

For:

Batch No.:

Best before:

Appearance:

Colour:

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Virus-RNA Isolation-Kit

Description

Column-based extraction of viral nucleic acids from a wide variety of starting samples (serums, plasma, cell culture supernatants, whole blood, cell-free body fluids, swabs, tissues, etc.). The kit contains carrier RNA and internal control DNA and RNA for QPCR tests. The isolated nucleic acids can be used immediately in all types of subsequent applications (such as PCR, QPCR, enzymatic applications).

Kit components

All components should be at room temperature before each use. Crystals that may have formed during delivery or storage can be dissolved by careful warming.

Important storage information:

- Lyophilized Proteinase K: at 4 °C
- Dissolved proteinase K: -20 °C
- (We recommend aliquoting the dissolved proteinase K in order to avoid frequent thawing, as this greatly reduces the activity of the enzyme)
- Lyophilized Carrier-Mix: at -20 °C
- Unused carrier mix stock solution: at -20°C
  (The solution should not be thawed and frozen more than 3 times; Therefore aliquot the stock solution and store at -20 °C)
- All other components: store in a dry place at room temperature

Virus-RNA Isolation-Kit

Kit components	250 Preparations
Lysis buffer SM	150 ml
Binding buffer WM	180 ml
Carrier Mix	for 3 x 1.25 ml working solution
RNase-free water	3 x 2 ml
Proteinase K	for 4 x 1.5 ml working solution
Washing buffer IT	70 ml
Washing buffer MT	36 ml
RNase-free Water	30 ml
Centrifugation column (purple)	5 x 50
Collection-Tubes 2.0 ml	25 x 50
Elution-Tubes 1.5 ml	5 x 50

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Important steps to take before you begin

- Add 96 – 99.8% ethanol to washing buffer IT and MT.
  Washing buffer IT
  Add **70 ml** of 96 - 99.8% ethanol to the bottle with washing buffer IT. Mix thoroughly and keep the bottle always tightly closed.

  Washing buffer MT
  Add **144 ml** of 96 – 99.8% ethanol to each bottle with washing buffer MT. Mix thoroughly and keep the bottle always tightly closed.

- Addition of ddH_2O to the lyophilized proteinase K.
  Dissolve proteinase K by adding 1.5 ml ddH_2O. Mix well (pipetting up and down several times), aliquot the solution and store at -20 °C.

- Addition of RNase-free H_2O to the lyophilized carrier mix.
  Dissolve the carrier mix by adding 1.25 ml RNase-free H_2O to each tube. Mix thoroughly by pipetting up and down. Aliquot the solution and store at -20 °C.

  Note: do not preheat RNase-free H_2O!

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Preparation of the Lysis Buffer SM/ Carrier Mix mixture

1. Add 1.25 ml of RNase-free water to each tube of Carrier Mix
2. Mix thoroughly by pipetting up and down!
3. Prepare the Lysis Buffer SM / Carrier Mix mixture according to the following table:

Component	**5** Preparations	**10** Preparations	**n** Preparations
Lyis buffer SM	2.7 ml	5.4 ml	540 µl per preparation
Carrier Mix	60 µl	120 µl	12 µl per preparation
Final volume	2.76 ml	5.52 ml	552 µl per preparation

Important notes:

- The carrier mix contains carrier RNA and internal control RNA.
- Immediately add the dissolved carrier mix to the lysis buffer SM!
- Freeze the unused carrier mix at -20 °C!
- Do not freeze and thaw carrier mix more than three times (store in aliquots)!
- The Lysis Buffer SM /Carrier Mix mixture is stable for one day at 4°C.

Components not included in the kit

- ddH_2O (RNAse free) to dissolve proteinase K
- 96 – 99.8% ethanol
  (only use pure ethanol, no methylated / denatured)
- 1.5 ml reaction tubes; Optional: 2.0 ml reaction tubes

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Important before you start working

- Preheat a thermomixer or a water bath to 70 ° C
- Temper RNase-free water to 70 °C (Attention: do not use for dissolving the carrier mix!)
- Make sure that Washing Buffer IT, Washing Buffer MT, Proteinase K and the Carrier Mix are prepared according to the instructions
- All centrifugation steps should be carried out at room temperature
- Avoid repeated freezing and thawing of the starting material

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Safety instructions

Please handle all materials and reagents included in the kit carefully. **Always wear gloves and avoid skin contact!**
In case of contact with eyes or skin, rinse immediately with plenty of water!
Please read each section carefully to ensure your own safety and ease of implementation. Follow all safety instructions in this brochure. Please also note instructions and information.

For single use
Do not use a component a second time!

Attention!

- Drinking or eating of the kit components are strictly prohibited!
- If the buffer bottles are damaged or leaking, wear gloves and safety goggles when disposing the bottles to avoid injury.
- This kit can be used with potentially infectious samples. Therefore, all liquid wastes must be considered as potentially infectious and must be handled and discarded according to local safety regulations.
- Please observe the federal, state and local safety and environmental regulations.
- Follow the usual precautions for working with extracted nucleic acids.
- All materials and reagents used for DNA or RNA isolation should be free of DNases and RNases.

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Attention!
Do not add any bleach or acidic components to the waste after sample preparation.

►►► Notes:
In case of an emergency: Medical information in English and German can be obtained 24 hours a day.
Telephone numbers of the Poison Information Centers:
Munich + 49 (0)89 19240
Vienna 01/406 43 43
Zurich + 41 44 251 51 51 (for Switzerland: 145)

For further information please request the safety data sheet.

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Virus-RNA Isolation-Kit

GHS-Classification

Component	Hazardous content	GHS Symbol	H Statements	P Statements	EUH
Lysis buffer SM	Guanidinium thiocyanate 25-50%	 Danger	302, 314, 412	101, 102, 103, 260, 303 + 361 + 353, 305 + 351 + 338, 310, 405, 501	032
Binding buffer WM	Guanidinium thiocyanate 25-50% Polyethylene glycol octyl phenol ether 25-50%	 Danger	302, 312, 314, 315, 318, 332, 411, 412	101, 102, 103, 260, 303 + 361 + 353, 305 + 351 + 338, 310, 405, 501	032
Washing buffer IT	Guanidinium-thiocyanate 50-100%	 Danger	302+ 312, 314, 332, 412	101, 102, 103, 260, 303 + 361 + 353, 305 + 351 + 338, 405, 501	032

H-Sätze

302 Harmful if swallowed.

312 Harmful in contact with skin.

314 Causes serious irritation of the skin and serious eye damage.

315 Causes skin irritation.

318 Causes serious eye damage.

332 Harmful by inhalation.

411 Toxic to aquatic life with long lasting effects

412 Harmful to aquatic organisms, with long-term effect

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P-Sätze

101	If medical advice is needed, have product container or label at hand.
102	Keep out of reach of children.
103	Read label before use.
260	Do not inhale dust / smoke / gas / mist / vapor / aerosol.
303 + 361 + 353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Wash skin [or shower] with water.
305 + 351 + 338	IF IN EYES: Rinse cautiously with water for several minutes. Remove any existing contact lenses if possible. Continue rinsing.
310	Call a POISON CENTER / doctor / ... immediately. Submit regulations for disposal.
405	Store locked up.
501	Dispose of contents/container in accordance with local and national legislation.

EUH-Sätze

032	Develops upon contact with acid very poisonous gases

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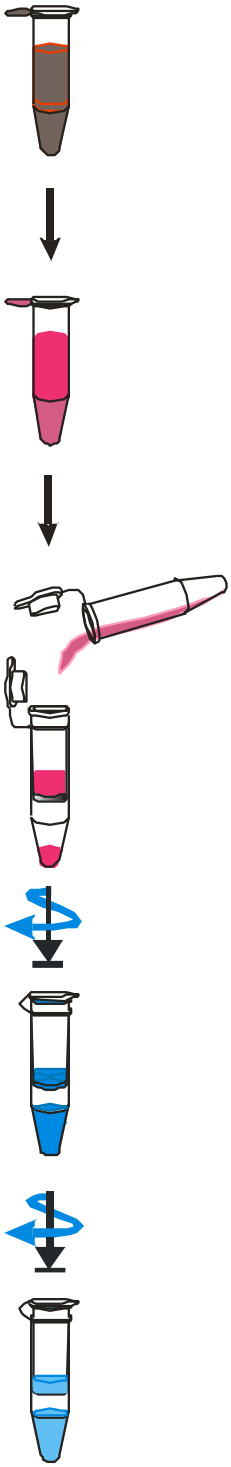
General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep 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 plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plasticware 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. Autoclaving alone will not inactivate many RNases completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0,1 % DEPC solution for 12 hours at 37°C and then it has to be autoclaved or heated 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 have to be prepared with DEPC-treated RNase-free ddH_2O.
- 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 plasticware employed for other applications which might introduce RNase contaminations in the RNA isolation.

Virus-RNA Isolation-Kit



Lysis of the starting material

Binding of the viral nucleic acids to the centrifugation filter

2x Washing of the bound DNA

Elution of the viral nucleic acids

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Protocol 1:

Isolation of viral RNA from serum, plasma, cell culture supernatants / media and other cell-free body fluids (up to 150 µl)

Important note: Before starting work, fill the required amount of RNase-free water into a 1.5 ml reaction tube and incubate it at 70 °C until the elution step!
Prepare the Lysis Buffer SM / Carrier Mix mixture as described above (page 5)!

1. **Open the 1.5 or 2.0 ml reaction vessel and add 450 µl Lysis Buffer SM / Carrier Mix. Add 150 µl of the sample and 20 µl proteinase K.** Mix thoroughly by vortexing several times for a total of 10 seconds and incubate for 10 minutes at room temperature, if possible with shaking (e.g. in a thermomixer).

   If continuous shaking is not possible, vortex the sample 3-4 times during incubation. After lysis, briefly centrifuge the reaction vessel to remove the condensate from the lid.
2. **Add 600 µl Binding Buffer WM** to the lysed sample and mix thoroughly by vortexing or pipetting up and down several times until a homogeneous solution is obtained.
   Note: The binding buffer is very viscous, so pipette carefully.

3. Place a **centrifugation column in a 2.0 ml collection tube**. **Apply 650 µl of the sample to the column** (purple). Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute.

Note: If the supernatant was not completely centrifuged through the membrane, centrifuge again at a higher speed or increase the centrifugation time.

Discard the collection tube with the flow through.

4. **Place the centrifugation column in a new 2.0 ml collection tube and transfer the remaining sample to the centrifugation column.** Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute.

   Note: If the supernatant was not completely centrifuged through the membrane, centrifuge again at a higher speed or increase the centrifugation time.

   Discard the collection tube with the flow through.
5. Place the centrifugation column in a new **2.0 ml collection tube**. Open the centrifugation column and add **500 µl wash buffer IT**. Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the collection tube with the flow through. Place the centrifugation column in a new 2.0 ml collection tube.
6. Open the centrifugation column and add **650 µl washing buffer MT**. Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the collection tube with the flow through.

   Place the centrifugation column in a **new 2.0 ml collection tube**.
7. Centrifuge at 10,000 x g (12,000 rpm) for 3 minutes to completely remove all ethanol residues. Discard the collection tube with the flow through. Place the centrifugation column in a **1.5 ml elution tube**.
8. Carefully open the centrifugation column and add **60 µl - 100 µl RNase-free water.** Incubate at room temperature for 2 minutes, then centrifuge at 8,000 x g (10,000 rpm) for 1 minute. For a maximum yield of RNA, two elution steps can be carried out with equal volumes of buffer (e.g. 30 µl + 30 µl).

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Note:

The RNA can also be eluted with lower or higher volumes of Elution buffer/ RNAase-free Water as indicated above. With lower volumes, the final concentration of the RNA increases. The extracted RNA should be stored at 4 °C - 8 °C. A temperature of - 22 °C to - 18 °C is recommended for long-term storage.

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Protocol 2:

Isolation of viral RNA from serum, plasma, cell culture supernatants / media and other cell-free body fluids (up to 300 µl)

Important note:

Before starting work, fill the required amount of RNase-free water into a 1.5 ml reaction tube and incubate it at 70 °C until the elution step!

Prepare the Lysis Buffer SM / Carrier Mix mixture as described above (page 5)!

1. Open **2.0 ml reaction vessel** and add **400 µl Lysis Buffer SM / Carrier Mix**. **Add 300 µl of the sample and 20 µl proteinase K**. Mix thoroughly by vortexing several times for a total of 10 seconds and incubate for 10 minutes at room temperature, if possible with shaking (e.g. in a thermomixer).
   If continuous shaking is not possible, vortex the sample 3-4 times during incubation. After lysis, briefly centrifuge the reaction vessel to remove the condensate from the lid.

2. **Add 700 µl Binding Buffer WM** to the lysed sample and mix thoroughly by vortexing or pipetting up and down several times until a homogeneous solution is obtained.
   Note: The binding buffer is very viscous, so pipette carefully.

3. Place a **centrifugation column in a 2.0 ml collection tube**. **Apply 650 µl of the sample to the column** (purple). Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute.

Note: If the supernatant was not completely centrifuged through the membrane, centrifuge again at a higher speed or increase the centrifugation time.
Discard the collection tube with the flow through.

4. **Place the centrifugation column** (purple) **in a new 2.0 ml collection tube and transfer the remaining sample to the centrifugation column.** Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute.
   Note: If the supernatant was not completely centrifuged through the membrane, centrifuge again at a higher speed or increase the centrifugation time.
   Discard the collection tube with the flow through.
5. Place the centrifugation column in a new **2.0 ml collection tube**. Open the centrifugation column and add **500 µl wash buffer IT**. Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the collection tube with the flow through. Place the centrifugation column in a new **2.0 ml collection tube**.
6. Open the centrifugation column and add **650 µl washing buffer MT**. Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the collection tube with the flow through.
   Place the centrifugation column in a **new 2.0 ml collection tube**.
7. Centrifuge at 10,000 x g (12,000 rpm) for 3 minutes to completely remove all ethanol residues. Discard the collection tube with the flow through. Place the centrifugation column in a **1.5 ml elution tube**.
8. Carefully open the centrifugation column and add **60 µl - 100 µl RNase-free water.** Incubate at room temperature for 2 minutes, then centrifuge at 8,000 x g (10,000 rpm) for 1 minute. For a maximum yield of RNA, two elution steps can be carried out with equal volumes of buffer (e.g. 30 µl + 30 µl).

Note:

The RNA can also be eluted with lower or higher volumes of Elution buffer/ RNAase-free Water as indicated above. With lower volumes, the final concentration of the RNA increases. The extracted RNA should be stored at 4 °C - 8 °C. A temperature of - 22 °C to - 18 °C is recommended for long-term storage.

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Protocol 3:

Isolation of viral RNA from tissue samples and biopsies (up to 20 mg)

Important note:

Before starting work, fill the required amount of RNase-free water into a 1.5 ml reaction tube and incubate it at 70 °C until the elution step!

Prepare the Lysis Buffer SM / Carrier Mix mixture as described above (page 5)!

1. Cut up to **20 mg tissue sample** into small pieces and place in **a 1.5 or 2.0 ml reaction tube**. Prepare a 10% (w / v) tissue buffer suspension by homogenizing the dissected tissue using commercial homogenizers (bead-based or other methods) in RNase-free water or PBS.
2. Centrifuge the reaction tube at maximum speed for 2 minutes to remove large particles. Use the clear, particle-free supernatant.
3. Open a new **1.5 ml reaction vessel** and add **450 µl Lysis Buffer SM / Carrier Mix**. Add **150 µl** of the sample and **20 µl proteinase K**. Mix thoroughly by vortexing several times for a total of 10 seconds and incubate for 10 minutes at room temperature, if possible with shaking (e.g. in a thermomixer). If continuous shaking is not possible, vortex the sample 3-4 times during incubation. After lysis, briefly centrifuge the reaction vessel to remove the condensate from the lid.
4. Add **450 µl Binding Buffer WM** to the lysed sample and mix thoroughly by vortexing or pipetting up and down several times until a homogeneous solution is obtained.

   Note: The binding buffer is very viscous, so pipette carefully.

5. Place a **centrifugation column in a 2.0 ml collection tube**. **Apply 650 µl of the sample to the column** (purple). Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute.
   Note: If the supernatant was not completely centrifuged through the membrane, centrifuge again at a higher speed or increase the centrifugation time.
   Discard the collection tube with the flow through.
6. **Place the centrifugation column** (purple) **in a new 2.0 ml collection tube and transfer the remaining sample to the centrifugation column.** Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute.
   Note: If the supernatant was not completely centrifuged through the membrane, centrifuge again at a higher speed or increase the centrifugation time.
   Discard the collection tube with the flow through.
7. Place the centrifugation column in a new **2.0 ml collection tube**. Open the centrifugation column and add **500 µl wash buffer IT**. Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the collection tube with the flow through. Place the centrifugation column in a new **2.0 ml collection tube**.
8. Open the centrifugation column and add **650 µl washing buffer MT**. Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the collection tube with the flow through.
   Place the centrifugation column in a **new 2.0 ml collection tube**.
9. Centrifuge at 10,000 x g (12,000 rpm) for 3 minutes to completely remove all ethanol residues. Discard the collection tube with the flow through. Place the centrifugation column in a **1.5 ml elution tube**.

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10. Carefully open the centrifugation column and add **60 µl - 100 µl RNase-free water.** Incubate at room temperature for 2 minutes, then centrifuge at 8,000 x g (10,000 rpm) for 1 minute. For a maximum yield of RNA, two elution steps can be carried out with equal volumes of buffer (e.g. 30 µl + 30 µl).

Note:

The RNA can also be eluted with lower or higher volumes of Elution buffer/ RNAase-free Water as indicated above. With lower volumes, the final concentration of the RNA increases. The extracted RNA should be stored at 4 °C - 8 °C. A temperature of - 22 °C to - 18 °C is recommended for long-term storage.

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Protocol 4:

Isolation of viral RNA from cheek swabs

Important note:

Before starting work, fill the required amount of RNase-free water into a 1.5 ml reaction tube and incubate it at 70 °C until the elution step!

Prepare the Lysis Buffer SM / Carrier Mix mixture as described above (page 5)!

1. Place the cheek swab in a **1.5 ml reaction tube** with physiological saline solution (0.9% NaCl). Incubate for 15 minutes at room temperature. Shake the swab thoroughly, squeeze it out and remove it from the tube.
   Continue working with **150 µl of the particle-free sample**.
2. Open a new **1.5 ml reaction vessel** and add **450 µl Lysis Buffer SM / Carrier Mix**. Add **150 µl** of the sample and **20 µl proteinase K**. Mix thoroughly by vortexing several times for a total of 10 seconds and incubate for 15 minutes at room temperature, if possible with shaking (e.g. in a thermomixer). If continuous shaking is not possible, vortex the sample 3-4 times during incubation. After lysis, briefly centrifuge the reaction vessel to remove the condensate from the lid.
3. Add **450 µl Binding Buffer WM** to the lysed sample and mix thoroughly by vortexing or pipetting up and down several times until a homogeneous solution is obtained.
   Note: The binding buffer is very viscous, so pipette carefully.

4. Place a **centrifugation column in a 2.0 ml collection tube**. **Apply 650 µl of the sample to the column** (purple). Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute.
   Note: If the supernatant was not completely centrifuged through the membrane, centrifuge again at a higher speed or increase the centrifugation time.
   Discard the collection tube with the flow through.
5. Place the centrifugation column in a new **2.0 ml collection tube**. Open the centrifugation column and add **500 µl wash buffer IT**. Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the collection tube with the flow through. Place the centrifugation column in a new **2.0 ml collection tube**.
6. Open the centrifugation column and add **650 µl washing buffer MT**. Close the lid and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the collection tube with the flow through.
   Place the centrifugation column in a **new 2.0 ml collection tube**.
7. Centrifuge at 10,000 x g (12,000 rpm) for 3 minutes to completely remove all ethanol residues. Discard the collection tube with the flow through. Place the centrifugation column in a **1.5 ml elution tube**.
8. Carefully open the centrifugation column and add **60 µl - 100 µl RNase-free water.** Incubate at room temperature for 2 minutes, then centrifuge at 8,000 x g (10,000 rpm) for 1 minute. For a maximum yield of RNA, two elution steps can be carried out with equal volumes of buffer (e.g. 30 µl + 30 µl).

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Note:

The RNA can also be eluted with lower or higher volumes of Elution buffer/ RNAase-free Water as indicated above. With lower volumes, the final concentration of the RNA increases. The extracted RNA should be stored at 4 °C - 8 °C. A temperature of - 22 °C to - 18 °C is recommended for long-term storage.

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Troubleshooting

Problem / possible cause	Comments and suggestions
Clogged spin column	
Insufficient lysis and / or too much starting material	**Extend** lysis. Increase centrifugation speed. After lysis, additional centrifugation to remove undissolved material. Use less starting material.
Low yield	
Insufficient lysis	**Extend** lysis. Use less starting material.
Incomplete elution	**Extend** the incubation time with RNase-free water or **repeat the elution step**. Perform elution with **higher volumes** of elution buffer.
Inadequate mixing with binding buffer WM	Ensure thorough mixing of the sample with binding buffer WM.
Low concentration of the virale RNA	
Too much elution buffer	Perform elution with low volumes of RNase free water.
No carrier RNA added	Add the carrier mix to the sample as described in the protocol.

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Technical Data

Shipment at room temperature

Storage: The Bio&SELL Virus-RNA Isolation-Kit should be stored dry at room temperature (14-25 °C). Under these conditions, it is at least stable for 12 months. Before each use, all components should be at room temperature. Crystals that have possibly formed during delivery or storage can be solved by careful warming.
Please note the further information on storage (page 2).

Precautionary Statement: This product should only be used by people who are familiar with laboratory applications. Standard laboratory protective clothing such as gowns, gloves and protective goggles should be worn. In the event of contact with skin or eyes, the affected areas should be washed or rinsed out immediately with water.

Application note: In certain countries, some applications for which this product can be used are protected by patents. Since no licenses are acquired through the purchase, it may be necessary to acquire the corresponding license rights, depending on the country of use and the application.

Quality control and technical support: All Bio&SELL GmbH products are subjected to extensive quality controls. This ensures that they will function properly when used as directed. We reserve the right to make changes to improve execution and design.

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Virus-RNA Isolation-Kit

Products for your research:

Reverse Transcription incl QRT-PCR (One-Step Mixe)

Bio&SELL
„One-Step RevTrans-qRT-PCR EvaGreen® No Rox“-Kit
Combines reverse transcription with quantitative real-time PCR based on the EvaGreen® dye.

Bio&SELL
„One-Step RevTrans-qRT-PCR EvaGreen® Rox“-Kit
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