minerva biolabs®

ExtractNow™ Virus RNA Swab Kit

Isolation of viral RNA from swabs

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

The ExtractNow™ Virus RNA Swab kit is developed for the isolation of viral RNA from swab specimens, especially tracheal swabs. Both direct isolation from swabs and extraction from swabs in physiological solution can be performed, following two optimized protocols. The purified RNA is free of contaminants and suitable for many downstream applications such as RT-PCR and RT-qPCR.

PRINCIPLE OF THE METHOD

The method is simple and consists of four general steps: (1) cell lysis, (2) selective binding of nucleic acids to spin columns, (3) removal of residual contaminants and inhibitors, and (4) elution of purified nucleic acids. The procedure does not require phenol/chloroform extraction and needs minimal hands-on time.

CONTENT

Each kit contains reagents for 250 extractions. The expiry date of the unopened package is marked on the package label. Store the lyophilized Proteinase K at +2 - +8 °C.
Store the lyophilized Carrier Reagent at ≤ –18 °C and all other components at room temperature (+18 to +25 °C). Before every use, ensure that all components are at room temperature. Dissolve any precipitates in the solutions by moderate warming.

Kit Component	250 extractions (Cat. No. 611-2250)
Spin columns	5 × 50 units
Collection tubes	5 × 50 units
Lysis Buffer B	150 ml
Carrier Reagent	for 3× working solution (add 1 ml of RNase-free water)
Proteinase K	for 2× working solution (add 1.5 ml of ddH_2O)
Wash Buffer D	36 ml (add 144 ml ethanol > 96 % abs. before first use)
RNase-free water	30 ml

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The ExtractNow™ Virus RNA Swab Kit contains reagents for isolating viral RNA from swabs. Additional consumables and equipment is supplied by the user:

- Ethanol > 96 % abs. (molecular biology grade)
- Ethanol 80 % (molecular biology grade)
- 1.5 ml and 2 ml tubes
- Microcentrifuge
- Heat block or thermomixer for 1.5 ml reaction tubes
- Pipettes with corresponding filter tips (10 and 1000 μl)
- ddH_2O to dissolve the Proteinase K
- 2-Propanol (molecular biology grade)

SPECIMEN

Avoid freeze-thaw-cycles of the starting material as it is detrimental to RNA integrity. In order to obtain best results it is also important not to overload spin columns.

- Always wear disposable laboratory gloves while handling the samples and reagents. Change gloves frequently.
- Keep samples as well as isolated RNA on ice.
- Use only RNase-free tubes and RNase-free filter tips.
- Do not handle any kind of cell cultures in the same laboratory where the RNA isolation will be conducted.
- Clean and decontaminate bench and pipettes (e.g. from nucleic acids with PCR Clean™) also using a RNase decontamination solution.
- All buffers and solutions should be prepared with RNase-free water.

PRECAUTIONS

The ExtractNow™ Virus RNA Swab Kit is for research use only. The kit should be used by trained laboratory staff only. All samples should be considered as potentially infectious and handled with all due care and attention. Always wear suitable lab coat, disposable gloves, and protective goggles. In case of contact, flush eyes or skin with water. Do not swallow components of the kit. Clean with suitable laboratory detergent and water, if any liquid is spilt. Remnants can be discarded according to local regulations.

IMPORTANT NOTES

⇒ Dissolve the Carrier Reagent with 1 ml RNase-free water (included in the kit) and mix thoroughly by pipetting. We recommend preparing aliquots of the dissolved Carrier Reagent. Do not freeze/thaw the Carrier Reagent more than three times.

⇒ Dissolve the Proteinase K with the 1.5 ml ddH_2O and mix thoroughly by pipetting. The dissolved Proteinase K must be stored at ≤ −18 °C. Repeated freeze/thaw cycles will reduce the enzyme activity. Therefore, we recommend preparing aliquots.

⇒ Make sure that ethanol has been added to Wash Buffer D. Do not use any alcohol other than ethanol as this will lead to inconsistent yields.

⇒ All the centrifugation steps should be carried out at room temperature.

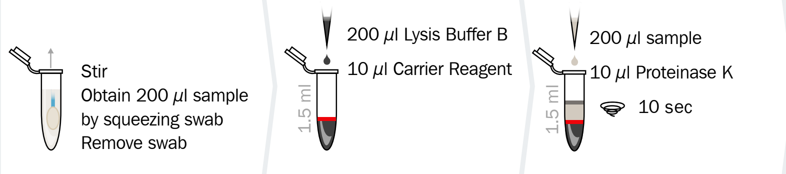
⇒ The reagents supplied should not be mixed with reagents from different lots but used as an integral unit. The reagents of the kit must not be used beyond shelf life.

⇒ Follow the exact protocol. Any deviation may affect the results.

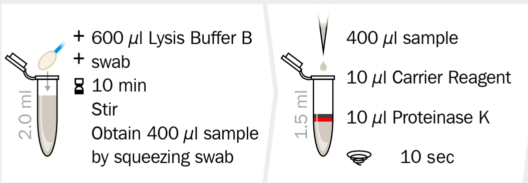
PROCEDURE - OVERVIEW

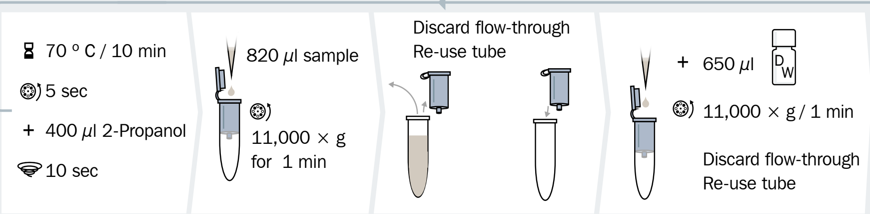
Isolation of viral RNA

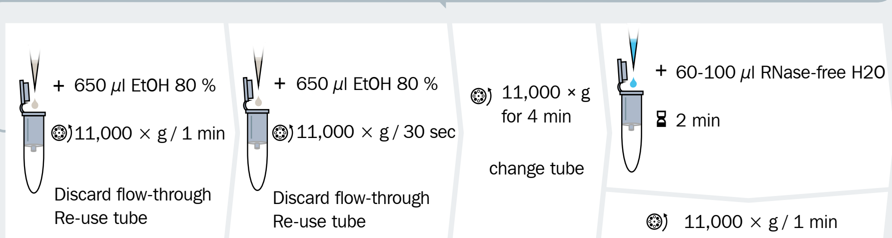
... from swabs in saline



... directly from swabs







+ add
vortex
incubate
centrifuge

D W Wash Buffer D

MB_SI_ExtractNow-Virus-RNA-Swab_01_EN

PROCEDURE - STEP BY STEP

Protocol 1: Isolation from swabs in physiological solution

⇒ Reconstitute Wash Buffer D, Carrier Reagent, and Proteinase K as described.
⇒ Set the heat block to 70 °C and pre-heat the needed volume of RNase-free water at 70 °C.

Step	Instruction
1.1.	Vigorously shake the swab in the storage tube containing physiological solution (or saline). Squeeze the swab against the tube walls to fully recover the liquid the swab is soaked in. Remove the swab and proceed to steps below with 200 µl of particle-free sample.
1.2.	Pipet 200 µl of Lysis Buffer B and 10 µl of Carrier Reagent into a 1.5 ml reaction tube.
1.3.	Add 200 µl of the sample and 10 µl of Proteinase K to each sample. Mix thoroughly by pulsed vortexing for 10 sec.
1.4.	Incubate at 70 °C for 10 min. We recommend the use of a thermomixer for a permanent shaking of the sample. Alternatively, vortex the sample 3 to 4 times during the incubation.
1.5.	Spin down the sample to collect the condensate and add 400 µl of 2-Propanol to the lysate. Mix by vortexing or by pipetting up and down several times. Note: Vortexing and vigorous mixing of the sample with 2-Propanol in order to obtain a homogeneous solution is essential.
1.6.	Place a spin column on a 2 ml collection tube. Apply the sample to the spin column. Close the cap and centrifuge at 11,000 x g for 1 min. Note: If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation.
1.7.	Discard the flow-through and re-use the collection tube by placing back the spin column.
1.8.	Add 650 µl Wash Buffer D, close the cap, and centrifuge at 11,000 x g for 1 min. Discard the flow-through and re-use the collection tube by placing back the spin column.
1.9.	Add 650 µl Ethanol 80 %, close the cap, and centrifuge at 11,000 x g for 1 min. Discard the flow-through and re-use the collection tube by placing back the spin column.
1.10.	Add 650 µl Ethanol 80 %, close the cap, and centrifuge at 11,000 x g for 30 sec. Discard the flow-through and re-use the collection tube by placing back the spin column.
1.11.	Centrifuge at 11,000 x g for 4 min to remove all traces of ethanol. Discard the collection tube and place the spin column on a new 1.5 ml tube.
1.12.	Add 60-100 µl of pre-heated RNase-free water and incubate at room temperature for 2 min.
1.13.	Centrifuge at 11,000 x g for 1 min. A second elution step (e.g. 30 µl + 30 µl) will increase the RNA yield. Note: The viral RNA can be eluted with a lower or a higher volume of RNase-free water (depending on the expected RNA yield). Elution with lower volumes of RNase-free water will increase the final concentration of viral RNA. Store the viral RNA at +2 - +8 °C or at ≤ −18 °C for long-term storage.

Protocol 2: Direct isolation from swabs

⇒ Reconstitute Wash Buffer D, Carrier Reagent, and Proteinase K as described.
⇒ Set the heat block to 70 °C and pre-heat the needed volume of RNase-free water at 70 °C.

2.1.	Transfer 600 µl of Lysis Buffer B into a 2.0 ml reaction tube.
2.2.	Incubate a freshly collected swab for 10 min into the 2.0 ml reaction tube with the Lysis Buffer B. Vigorously shake the swab and squeeze it against the tube walls in order to fully recover the liquid the swab is soaked in. Remove the swab and proceed to steps below with 400 µl of particle-free sample.
2.3.	Transfer 400 µl of the sample to a 1.5 ml reaction tube and add 10 µl of Carrier Reagent and 10 µl of Proteinase K to each sample. Mix thoroughly by pulsed vortexing for 10 sec.
2.4.	Incubate at 70 °C for 10 min. We recommend the use of a thermomixer for a permanent shaking of the sample. Alternatively, vortex the sample 3 to 4 times during the incubation.
2.5.	Spin down the sample to collect the condensate and add 400 µl of 2-Propanol to the lysate. Mix by vortexing or by pipetting up and down several times. Note: Vortexing and vigorous mixing of the sample with 2-Propanol in order to obtain a homogeneous solution is essential.
2.6.	Place a spin column on a 2 ml collection tube. Apply the sample to the spin column. Close the cap and centrifuge at 11,000 x g for 1 min. Note: If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation.
2.7.	Discard the flow-through and re-use the collection tube by placing back the spin column.
2.8.	Add 650 µl Wash Buffer D, close the cap, and centrifuge at 11,000 x g for 1 min. Discard the flow-through and re-use the collection tube by placing back the spin column.
2.9.	Add 650 µl Ethanol 80 %, close the cap, and centrifuge at 11,000 x g for 1 min. Discard the flow-through and re-use the collection tube by placing back the spin column.
2.10.	Add 650 µl Ethanol 80 %, close the cap, and centrifuge at 11,000 x g for 30 sec. Discard the flow-through and re-use the collection tube by placing back the spin column.
2.11.	Centrifuge at 11,000 x g for 4 min to remove all traces of ethanol. Discard the collection tube and place the spin column on a new 1.5 ml tube.
2.12.	Add 60-100 µl of pre-heated RNase-free water and incubate at room temperature for 2 min.
2.13.	Centrifuge at 11,000 x g for 1 min. A second elution step (e.g. 30 µl + 30 µl) will increase the RNA yield. Note: The viral RNA can be eluted with a lower or a higher volume of RNase-free water (depending on the expected RNA yield). Elution with lower volumes of RNase-free water will increase the final concentration of viral RNA. Store the viral RNA at +2 - +8 °C or at ≤ –18 °C for long-term storage.

APPENDIX

Limited Product Warranty
This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from of the use, the results of use, or the inability to use this product.

Trademarks
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RELATED PRODUCTS

DNA Extraction kits

56-1010/-1050/	Venor®GeM Sample Preparation Kit	10/50 extractions
602-1010/-1050	ExtractNow™ Blood DNA Mini kit	10/50 extractions
603-1010/-1050	ExtractNow™ RNA Mini kit	10/50 extractions
604-1010/-1050	ExtractNow™ Cleanup kit	10/50 extractions
605-1010/-1050	ExtractNow™ Plasmid Mini kit	10/50 extractions
606-1010/-1050	ExtractNow™ Virus DNA/RNA kit	10/50 extractions
611-1010/-1050/-1250	ExtractNow™ Virus RNA kit	10/50/250 extractions

PCR & RT-PCR Mix

192-0025/-0100/-0250	ConviFlex™ RT-Taq Mix, one-step mix with Taq polymerase and reverse transcriptase for RT-qPCR	25/100/250 reactions
191-025/-100/-250	ConviFlex™ DNAmp Mix, one step mix with Taq polymerase for conventional and qPCR	25/100/250 reactions

MB Taq Polymerase

53-0050/-0100/-0200/-0250	MB Taq DNA Polymerase (5 U/µl)	50/100/200/250 units
53-1050/-1100/-1200/-1250	MB Taq DNA Polymerase (1 U/µl)	50/100/200/250 units

PCR Clean™

15-2025/2200/2500	DNA Decontamination Reagent, spray bottle/refill canister	250 ml/5 l
15-2001/-2002	Wipes in a dispenser box/ refill packs	50 wipes/5 × 50 wipes

LabClean™

15-4100	DNA Decontamination Reagent	1 l

WaterShield™

15-3015/3020/3050	Water Disinfection Additive for incubators and water baths, 200x concentrate	30 × 5 ml/3 × 50 ml/500 ml

ZellShield®

13-0050/-0150	Contamination Prevention Reagent 100× concentrate	50 ml/ 3×50 ml

Mycoplasma Off™

15-1000/-5000	Surface Disinfectant Spray, spray bottle/ refill canister	1 l/ 5 l
15-1001/-5001	Surface Disinfectant Wipes in dispenser box/refill pack	50 wipes/5 x 50 wipes

PCR Cycler Validation

57-2102/-2103	PCR Cycler Check™ Advance/OneStep	6 strips, 8 vials each/100 reactions
57-2202	qPCR Cycler Check™	100 reactions

SwabUp™ Lab Monitoring Kits

181-0010/-0050	Sample collection and DNA extraction	10/50 samples
182-0010/-0050	Sample collection, DNA extraction and PCR system	10/50 samples

Contamination Control Kits for conventional PCR

11-7024/-7048/-7096/-7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 reactions
11-8025/-8050/-8100/-8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 reactions
12-1025/-1050/-1100/-1250	Onar® Bacteria Detection Kit	25/50/100/250 reactions

Contamination Control Kits for qPCR

11-9025/-9100/-9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 reactions
11-91025/-91100/-91250	Venor®GeM qOneStep Mycoplasma Detection Kit	25/100/250 reactions

Sample Preparation

56-1010/-1050/-1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions

Mycoplasma Elimination

10-0200/-0500/-1000	Mynox® Mycoplasma Elimination Reagent	2/5/10 treatments
10-0201/-0501/-1001	Mynox® Gold Mycoplasma Elimination Reagent	2/5/10 treatments

Minerva Biolabs GmbH
Schkopauer Ring 13
D-12681 Berlin, Germany

www.minerva-biolabs.com
Ordering: order@minerva-biolabs.com
Support: support@minerva-biolabs.com

USA & Canada

Minerva Biolabs Inc.
1 Jill Ct., Building 16, Unit 10
Hillsborough, NJ 08844
USA

www.minervabiolabs.us
Ordering: order@minervabiolabs.us
Support: help@minervabiolabs.us

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