ExtractNow™ Virus RNA Kit

Isolation of viral RNA from a variety of starting materials

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 kit is developed for isolating viral RNA from a broad range of starting material such as cell-free biological fluids (plasma, serum, urine, liquor, and cell culture supernatant) but also tissue and swab specimen. Up to 300 µl of fluid and up to 20 mg of solid material can be used. The purified nucleic acids are free of contaminants and suitable for many downstream applications such as PCR and RT-PCR.

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 handling time.

CONTENT

Each kit contains reagents for 10, 50 or 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	10 extractions (Cat. No. 611-1010)	50 extractions (Cat. No. 611-1050)	250 extractions (Cat. No. 611-1250)
Spin columns	10 units	50 units	5 X 50 units
Collection tubes	50 units	5 X 50 units	25 x 50 units
Lysis Buffer D	8 ml	30 ml	150 ml
Binding Buffer E	8 ml	40 ml	180 ml
Carrier Reagent	for 1 X working solution (add 1.25 ml of RNAse-free water)	for 1 X working solution (add 1.25 ml of RNAse-free water)	for 3 X working solution (add 1.25 ml of RNAse-free water)
Proteinase K	for 1 X working solution (add 0.3 ml of ddH_2O)	for 1 X working solution (add 1.5 ml of ddH_2O)	for 4 X working solution (add 1.5 ml of ddH_2O)
Wash Buffer C	3 ml (add 3 ml ethanol > 96 % abs. before first use)	15 ml (add 15 ml ethanol > 96 % abs. before first use)	70 ml (add 70 ml ethanol > 96 % abs. before first use)
Wash Buffer D	2 ml (add 8 ml ethanol > 96 % abs. before first use)	8 ml (add 32 ml ethanol > 96 % abs. before first use)	36 ml (add 144 ml ethanol > 96 % abs. before first use)
RNAse-free water	2 ml	6 ml	30 ml 3 X 2 ml

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

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The ExtractNow™ Virus RNA kit contains reagents for isolating viral RNA from various sources. Additional consumables and equipment is supplied by the user:

- Ethanol > 96 % abs.
- 1.5 ml and 2 ml tubes
- Microcentrifuge
- Heat block or thermomixer for 1.5 ml and 2 ml reaction tubes
- Pipettes with corresponding filter tips (100 and 1000 µl)
- ddH_2O to dissolve the Proteinase K
- PBS or saline (0.9 % NaCl)

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. The maximum amounts of starting material are:

- 150 or 300 µl fluid samples
- up to 20 mg of tissue samples

Generally, RNA is less stable than DNA and particularly sensitive to degradation by RNAses. It is therefore essential to follow these recommendations:

- 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 bench and pipettes with a RNAse decontamination solution (e.g. PCR Clean™).
- All buffers and solutions should be prepared with RNAse-free water.

PRECAUTIONS

The ExtractNow™ Virus RNA 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. This kit can be disposed of as municipal waste according to local guidelines.

IMPORTANT NOTES

⇒ Dissolve the Proteinase K with the given volume of ddH_2O and mix thoroughly by pipetting. Dissolved Proteinase K must be stored at ≤–18 °C. Repeated freeze/thaw cycles will reduce the enzyme activity. We therefore recommend to prepare aliquots.

⇒ Dissolve the Carrier Reagent with 1.25 ml RNAse-free water (included in the kit) and mix thoroughly by pipetting. We recommend to prepare aliquots of the dissolved Carrier Reagent. Do not freeze/thaw the Carrier Reagent more than three times.

Component	Volume per 10 samples	Volume per sample
Lysis Buffer D	5.4 ml	540 µl x sample
Carrier Reagent	120 µl	12 µl x sample
Final Volume	5.52 ml	552 µl

⇒ Ensure that ethanol was added to Wash Buffer C and D. Do not use other alcohol apart from ethanol as it will lead to inconsistent yields.

⇒ 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 - STEP BY STEP

Protocol 1: Isolation of viral RNA from fluids up to 150 µl

⇒ Prepare Lysis Buffer D/Carrier Reagent mix as described
⇒ Reconstitute Wash Buffers C and D, and Proteinase K as described.
⇒ Set the heat block to 70 °C. Equilibrate the needed volume of RNAse-free water at 70°C.

1.1.	Pipet 450 µl of the Lysis Buffer D/Carrier Reagent mix into a 1.5 ml reaction tube. Add 150 µl of the sample and 20 µl of Proteinase K. Mix thoroughly by pulsed vortexing for 10 sec.
1.2.	Incubate at room temperature 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.
1.3.	Spin down the sample and add 600 µl of Binding Buffer E to the lysate. Mix by vortexing or by pipetting up and down several times. Note: It is important that the sample and Binding Buffer E are mixed vigorously to obtain a homogeneous solution.
1.4.	Pipet 650 µl of the sample to a spin column placed on a collection tube. Close the cap and centrifuge at 10,000 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.
1.5.	Discard the collection tube and place the spin column on a new collection tube.
1.6.	Add 500 µl Wash Buffer C and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column on a new collection tube.
1.7.	Add 650 µl Wash Buffer D and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column on a new collection tube.
1.8.	Centrifuge at max. speed for 3 min to remove all traces of ethanol. Discard the collection tube and place the spin column on a new 1.5 ml tube.
1.9.	Add 60-100 µl of pre-heated RNAse-free water and incubate at room temperature for 2 min.
1.10.	Centrifuge at 8000 x g for 1 min. A second elution step will increase the yield of extracted RNA. Note: The viral RNA can be eluted with a lower or a higher volume of RNAse-free water (depends on the expected yield of RNA). Elution with lower volumes of RNAsefree water will increase the final concentration of viral RNA. Store the viral RNA at +2 - 8 °C or at ≤–18 °C for long time storage.

Protocol 2: Isolation of viral RNA from fluids up to 300 µl

⇒ Prepare Lysis Buffer D/Carrier Reagent mix as described
⇒ Reconstitute Wash Buffers C and D, and Proteinase K as described.
⇒ Set the heat block to 70 °C. Equilibrate the needed volume of RNAse-free water at 70°C.

2.1.	Pipet 400 µl of the Lysis Buffer D/Carrier Reagent mix into a 1.5 ml reaction tube. Add 300 µl of the sample and 20 µl of Proteinase K. Mix thoroughly by pulsed vortexing for 10 sec.
2.2.	Incubate at room temperature 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.
2.3.	Spin down the sample and add 700 µl of Binding Buffer E to the lysate. Mix by vortexing or by pipetting up and down several times. Note: It is important that the sample and Binding Buffer E are mixed vigorously to obtain a homogeneous solution.
2.4.	Pipet 650 µl of the sample to a spin column placed on a collection tube. Close the cap and centrifuge at 10,000 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.
2.5.	Discard the collection tube and place the spin column on a new collection tube.
2.6.	Place the spin column on a new collection tube and add the rest of the sample. Close the cap and centrifuge at 10,000 x g for 1 min.
2.7.	Discard the collection tube and place the spin column on a new collection tube.
2.8.	Add 500 µl Wash Buffer C and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column on a new collection tube.
2.9.	Add 650 µl Wash Buffer D and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column on a new collection tube.
2.10.	Centrifuge at max. speed for 3 min to remove all traces of ethanol. Discard the collection tube and place the spin column on a new 1.5 ml tube.
2.11	Add 60-100 µl of pre-heated RNAse-free water and incubate at room temperature for 2 min.
2.12..	Centrifuge at 8000 x g for 1 min. A second elution step will increase the yield of extracted RNA. Note: The viral RNA can be eluted with a lower or a higher volume of RNAse-free water (depends on the expected yield of RNA). Elution with lower volumes of RNAsefree water will increase the final concentration of viral RNA. Store the viral RNA at +2 - 8 °C or at ≤–18 °C for long time storage.

Protocol 3: Isolation of viral RNA from tissue/biopsy material (max. 20 mg)

⇒ Prepare Lysis Buffer D/Carrier Reagent mix as described
⇒ Reconstitute Wash Buffers C and D, and Proteinase K as described.
⇒ Set the heat block to 70 °C. Equilibrate the needed volume of RNAse-free water at 70°C.

3.1.	Cut up to 20 mg of the sample into small pieces. Place the sample into PBS or RNAse-free water at a proportion of 1 to 10 (w/v). Homogenize the sample with an appropriate tool (e.g. bead homogenizer).
3.2.	Centrifuge the homogenized suspension at max. speed for 2 min to pellet any particles. Use the supernatant for the following steps.
3.3.	Pipet 450 µl of the Lysis Buffer D/Carrier Reagent mix into a 1.5 ml reaction tube. Add 150 µl of the sample and 20 µl of Proteinase K. Mix thoroughly by pulsed vortexing for 10 sec.
3.4.	Incubate at room temperature 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.
3.5.	Spin down the sample and add 450 µl of Binding Buffer E to the lysate. Mix by vortexing or by pipetting up and down several times. Note: It is important that the sample and Binding Buffer E are mixed vigorously to obtain a homogeneous solution.
3.6.	Pipet 650 µl of the sample to a spin column placed on a collection tube. Close the cap and centrifuge at 10,000 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.
3.7.	Discard the collection tube and place the spin column on a new collection tube.
3.8.	Place the spin column on a new collection tube and add the rest of the sample. Close the cap and centrifuge at 10,000 x g for 1 min.
3.9.	Discard the collection tube and place the spin column on a new collection tube.
3.10.	Add 500 µl Wash Buffer C and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column on a new collection tube.
3.11.	Add 650 µl Wash Buffer D and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column on a new collection tube.
3.12.	Centrifuge at max. speed for 3 min to remove all traces of ethanol. Discard the collection tube and place the spin column on a new 1.5 ml tube.
3.13.	Add 60-100 µl of pre-heated RNAse-free water and incubate at room temperature for 2 min.
3.14.	Centrifuge at 8000 x g for 1 min. A second elution step will increase the yield of extracted RNA. Note: The viral RNA can be eluted with a lower or a higher volume of RNAse-free water (depends on the expected yield of RNA). Elution with lower volumes of RNAsefree water will increase the final concentration of viral RNA. Store the viral RNA at +2 - 8 °C or at ≤–18 °C for long time storage.

Protocol 4: Isolation of viral RNA from swab material

⇒ Prepare Lysis Buffer D/Carrier Reagent mix as described
⇒ Reconstitute Wash Buffers C and D, and Proteinase K as described.
⇒ Set the heat block to 70 °C. Equilibrate the needed volume of RNAse-free water at 70°C.

4.1.	Place the swab into a 1.5 ml reaction tube containing 500 µl saline solution (0.9 % NaCl). Incubate at room temperature for 15 min. Stir the swab vigorously and remove the swab. Ensure to squeeze the liquid out of the tip of the swab. Proceed with 150 µl of the (particle-free) liquid.
4.2.	Pipet 450 µl of the Lysis Buffer D/Carrier Reagent mix into a 1.5 ml reaction tube. Add 150 µl of the sample and 20 µl of Proteinase K. Mix thoroughly by pulsed vortexing for 10 sec.
4.3.	Incubate at room temperature for 15 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.
4.4.	Spin down the sample and add 450 µl of Binding Buffer E to the lysate. Mix by vortexing or by pipetting up and down several times. Note: It is important that the sample and Binding Buffer E are mixed vigorously to obtain a homogeneous solution.
4.5.	Pipet 650 µl of the sample to a spin column placed in a collection tube. Close the cap and centrifuge at 10,000 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.
4.6.	Discard the collection tube and place the spin column in a new collection tube.
4.7.	Place the spin column on a new collection tube and add the rest of the sample. Close the cap and centrifuge at 10,000 x g for 1 min.
4.8.	Discard the collection tube and place the spin column on a new collection tube.
4.9.	Add 500 µl Wash Buffer C and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column in a new collection tube.
4.10.	Add 650 µl Wash Buffer D and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column on a new collection tube.
4.11.	Centrifuge at max. speed for 3 min to remove all traces of ethanol. Discard the collection tube and place the spin column on a new 1.5 ml tube.
4.12.	Add 60-100 µl of pre-heated RNAse-free water and incubate at room temperature for 2 min.
4.13.	Centrifuge at 8000 x g for 1 min. A second elution step will increase the yield of extracted RNA. Note: The viral RNA can be eluted with a lower or a higher volume of RNAse-free water (depends on the expected yield of RNA). Elution with lower volumes of RNAsefree water will increase the final concentration of viral RNA. Store the viral RNA at +2 - 8 °C or at ≤–18 °C for long time 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		
56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50 extractions
602-1010/1050/1200	ExtractNow™ Blood DNA Mini kit	10/50 extractions
603-1010/1050/1200	ExtractNow™ RNA Mini kit	10/50 extractions
604-1010/1050/1200	ExtractNow™ Cleanup kit	10/50 extractions
605-1010/1050/1200	ExtractNow™ Plasmid Mini kit	10/50 extractions
606-1010/1050/1200	ExtractNow™ Virus DNA/RNA kit	10/50 extractions
PCR Clean™		
15-2025/2200/2500	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4 × 500 ml
15-2001	Wipes	50 wipes in a dispenser box
15-2002	Wipes, refill packs	5 × 50 wipes in a bag
LabClean™		
15-4100	Molecular Microbiology Lab Cleaner	1 liter
WaterShield™		
15-3015/3020/3050	Water Disinfection Additive for incubators and water baths, 200x concentrate	30 × 5 ml/3 × 50 ml/500 ml
Mycoplasma Off™		
15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5l
15-1001	Surface Disinfectant Wipes in dispenser box	50 wipes
15-5001	Surface Disinfectant Wipes, refill pack	5 x 50 wipes
ConviFlex™ RT-Taq Mix		
1192-0025/-0100/-0250	RT-PCR Mix with Taq polymerase and reverse transcriptase	25/100/250 reactions

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