ExtractNow™ Sewage Water DNA/RNA Kit

Isolation of viral and bacterial DNA/RNA from waste water

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Contents

1 Introduction

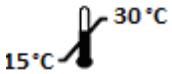
1.1 Intended use

The ExtractNow™ Sewage Water DNA/RNA Virus Kit has been designed for the isolation of DNA or RNA from sewage water samples.
The kit allows the isolation of DNA/RNA from viruses, bacteria and free-circulating DNA (e.g. plasmid associated DNA coding for bacterial resistance) in sewage water sediment as well as from the cleared supernatant after spinning down the sediment. The latest data shows that nucleic acids could be detected in the sediment and the supernatant from sewage water samples.
The kit contains three protocols. The first protocol allows the isolation of nucleic acids from sewage, the second from the corresponding supernatant and the third protocol the simultaneously and parallel isolation of nucleic acids from sewage sediment and the corresponding cleared supernatant.
The kit combines a unique patented technology for enrichment of biomolecules with a very efficient extraction of DNA and RNA using magnetic particles.
The extraction process starts with 20 ml of sewage water sample. After sedimentation of solid material 10 ml of the resulting supernatant and the resulting sediment (appr. 100 – 200 mg) will be used for the extraction of DNA and RNA.
Depending on the respective protocol the supernatant and the sediment alone or both combined could be used for isolating nucleic acids.

CONSULT INSTRUCTION FOR USE
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual

For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	REF Catalogue number.
	Content Contains sufficient reagents for <N> reactions.
	Storage conditions Store at room temperature or shown conditions respectively.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
	Lot number The number of the kit charge.
	Manufactured by Contact information of manufacturer.
	For single use only Do not use components for a second time.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → „Notes on the use of this manual” p. 3).
- Work steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully prior to guarantee your own safety and a trouble-free operation.
Follow all the safety instructions explained in the manual, as well as all messages and information that are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, immediately flush eyes or skin with a large amount of water.

FOR SINGLE USE ONLY!
This kit is made for single use only!

ATTENTION!
Don’t eat or drink components of the kit!
The kit shall only be handled by educated personnel in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.
Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!
Do not add bleach or acidic components to the waste after sample preparation!

NOTE

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Center Freiburg, Germany

Phone: +49 761 19240

For more information on GHS classification and the Safety Data Sheet (SDS) please dial +49 30 20004370.

3 Storage conditions

All kit components are shipped at ambient temperature.

Store lyophilized and dissolved Proteinase K, Carrier Reagent, Beads M and Enrichment Reagent A at 4 °C to 8 °C.

All other components of the ExtractNow™ Sewage Water DNA/RNA Kit should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions dissolve these precipitates by careful warming.

For further information see chapter “Kit components” (→ p. 7).

4 Functional testing and technical assistance

The Minerva Biolabs GmbH guarantees the correct function of the kit for applications as described in the manual. This kit has been produced and tested in an ISO 13485 certified facility.
We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the ExtractNow™ Sewage Water DNA/RNA Kit or other Minerva Biolabs GmbH products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 30 20004370. For other countries please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual, (→ “Product specifications“ p. 8). Since the performance characteristics of Minerva Biolabs GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of Minerva Biolabs GmbH kits using other protocols than those described below. Minerva Biolabs GmbH kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA’ 88 regulations in the U.S. or equivalents in other countries.
All products sold by Minerva Biolabs GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 50
REF	612-1050
Beads M	2 ml
Proteinase K	for 1 x 1.5 ml working solution
Carrier Reagent	for 1 x 1.0 ml working solution
Lysis Buffer E	50 ml
Binding Buffer C	50 ml
Washing Buffer C (conc.)	60 ml
Washing Buffer D (conc.)	36 ml
Enrichment Reagent A	5 x 1.2 ml
Enrichment Reagent B	5 ml
Manual	1

6.2 Components not included in the kit

- 50 ml tubes
- 2.0 ml or 1.5 ml reaction tubes
- ddH_2O for dissolving Proteinase K
- 96–99.8 % ethanol, non-denatured or methylated
- 10 mM Tris-HCl pH 8.0-8.5 or RNase-free water for elution step
- 1 x PBS
  (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)

7 Product specifications

1. Starting material:

- 20 ml sewage water

2. Time for isolation:

- Approximately 60 minutes including all steps

8 Initial steps before starting

- Add 1.5 ml ddH_2O to lyophilized Proteinase K.
- Add 1.0 ml ddH_2O to lyophilized Carrier Reagent, mix thoroughly and store as described above.
- Add 60 ml ethanol to 60 ml Washing Buffer C (conc.), mix thoroughly and store as described above.
- Add 144 ml ethanol to 36 ml Washing Buffer E (conc.), mix thoroughly and store as described above.
- Heat thermal mixer or water bath to 60 °C (later to 50 °C).
- Centrifugation steps should be carried out at room temperature.

9 Protocol 1: Isolation of DNA/RNA from sewage sediment

9.1 Preliminary steps before the separation of sediment from supernatant

1. Transfer 20 ml of sewage water sample into a 50 ml tube. Centrifuge at 5.000 rpm for 10 minutes.

2. Remove the clear supernatant as complete as possible and proceed further steps with the sediment.

9.2 Isolation of DNA/RNA from the sediment

1. Add 250 µl 1 x PBS to the tube containing the sediment. Resuspend the sediment completely and transfer the resuspended sediment into a 1.5 ml tube.

NOTE
The amount of sediment should be appr. 100 – 200 mg. If the amount is > 200 mg please reduce the amount of sediment to 100 – 200 mg.

2. Centrifuge the reaction tube at max. speed for 3 minutes.

3. Transfer the clear supernatant of the sample (max. 200 µl) into a new 1.5 ml reaction tube. Add 300 µl Lysis Buffer E, 20 µl Proteinase K and 10 µl Carrier Reagent. Vortex shortly. Place the reaction tube into a thermal mixer and incubate under continuous shaking for 15 minutes at 60°C.

4. Add 450 µl Binding Buffer C and 20 µl Beads M to the lysed sample.

NOTE
Vortex the Beads M for 1 minute before use! The Binding Buffer C is viscously, please pipette carefully.

5. Mix the sample completely by vortexing for 15 seconds. Incubate the reaction tube at room temperature for 5 minutes for the binding of the nucleic acids to the magnetic particles.

6. Place the reaction tube in a magnetic rack or another magnetic particle separation equipment. Separate the beads from the supernatant and remove the supernatant as complete as possible using a pipet tip.

7. Add 500 µl Washing Buffer C and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of the beads and remove the Washing Buffer C.

8. Add 500 µl Washing Buffer C and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of beads and remove the Washing Buffer C.

9. Add 750 µl Washing Buffer D and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the Washing Buffer D.

10. Add 750 µl Washing Buffer D and wash the magentic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the Washing Buffer D.

NOTE
After the last washing step remove Washing Buffer D as complete as possible!

11. Place the opened reaction tube with the magnetic beads in a thermal mixer at 50 °C for 5 minutes.

NOTE
The drying step is important for all following downstream application. The ethanol must be removed completely!

12. Add 40 µl – 100 µl RNase-free water or 10 mM Tris-HCl pH 8.0-8.5, resuspend the magnetic particles completely and incubate at 50°C in a thermal mixer under continuous shaking for 5 minutes.

NOTE
The elution volume depends on expected amount of target nucleic acid

13. Perform magnetic separation of beads. Transfer the eluted DNA/RNA into a new 1.5 ml reaction tube.

10 Protocol 2: Isolation of DNA/RNA from sewage supernatant

10.1 Preliminary steps

1. Transfer 20 ml of sewage water sample into a 50 ml tube. Centrifuge at 5.000 rpm for 10 minutes.

2. Transfer 10 ml clear supernatant into a new 50 ml tube.

10.2 Isolation of viral DNA/RNA from supernatant

1. Add 100 µl Enrichment Reagent 1. Shake the tube shortly.

2. Add 100 µl Enrichment Reagent 2. Shake the tube shortly. Incubate at room temperature for 10 minutes.

3. Centrifuge the tube at least at 5.000 rpm for 10 minutes, open the tube and remove the supernatant carefully as much as possible.

NOTE
Don’t remove the pellet.

4. Add 5 ml RNAse-free Water to the tube, invert the tube three times and centrifuge at least at 5.000 rpm for 5 minutes.

5. Open the tube and remove the supernatant carefully as much as possible.

NOTE
Don’t remove the pellet.

6. Add 500 µl Lysis Buffer E. Resuspend the pellet completely by pipetting up and down and transfer the resuspended pellet into a new 1.5 ml reaction tube. Add 20 µl Proteinase K and 10 µl Carrier Reagent. Vortex shortly. Place the reaction tube into a thermal mixer and incubate under continuous shaking for 15 minutes at 60°C.

7. Add 450 µl Binding Buffer C and 20 µl Beads M to the lysed sample.

NOTE
Vortex the Beads M for 1 minute before use! The Binding Buffer C is viscously, please pipette carefully.

8. Mix the sample completely by vortexing for 15 seconds. Incubate the reaction tube at room temperature for 5 minutes for the binding of the nucleic acids to the magnetic particles.

9. Place the reaction tube in a magnetic rack or another magnetic particle separation equipment. Separate the beads from the supernatant and remove the supernatant as complete as possible using a pipet tip.

10. Add 500 µl Washing Buffer C and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of the beads and remove the Washing Buffer C.

11. Add 500 µl Washing Buffer C and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of beads and remove the Washing Buffer C.

12. Add 750 µl Washing Buffer D and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the Washing Buffer D.

13. Add 750 µl Washing Buffer D and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the Washing Buffer D.

NOTE
After the last washing step remove Washing Buffer D as complete as possible!

14. Place the opened reaction tube with the magnetic beads in a thermal mixer at 50 °C for 5 minutes.

NOTE
The drying step is important for all following downstream application. The ethanol must be removed completely!

15. Add 40 µl – 100 µl RNase-free water or 10 mM Tris-HCl pH 8.0-8.5, resuspend the magnetic particles completely and incubate at 50°C in a thermal mixer under continuous shaking for 5 minutes.

NOTE
The elution volume depends on expected amount of target nucleic acid

16. Perform magnetic separation of beads. Transfer the eluted DNA/RNA into a new 1.5 ml reaction tube.

11 Protocol 3: Simultaneously isolation of DNA/RNA from sewage water sediment and supernatant

11.1 Preliminary steps

1. Transfer 20 ml of sewage water sample into a 50 ml tube. Centrifuge at 5.000 rpm for 10 minutes.

2. Transfer the clear supernatant complete as possible into a new 50 ml tube. Don`t through away the supernatant!!!

11.2 Simultaneously Isolation of DNA/RNA from sediment and supernatant

1. Add 250 µl 1 x PBS to the tube containing the sediment. Resuspend the sediment completely and transfer the resuspended sediment into a 1.5 ml reaction tube.

NOTE
The amount of sediment should be appr. 100 – 200 mg. If the amount is > 200 mg please reduce the amount of sediment to 100 – 200 mg.

2. Centrifuge the reaction tube at max. speed for 3 minutes.

3. Transfer the clear supernatant of sample (max. 200 µl) into a new 1.5 ml reaction tube. Let stay the reaction tube for further processing and proceed with the supernatant.

4. Add 10 ml of the supernatant from the preliminary step for separating sediment and supernatant form the initial sample into a new 15 ml tube. Add 100 µl Enrichment Reagent 1. Shake the tube shortly.

5. Add 100 µl Enrichment Reagent 2. Shake the tube shortly. Incubate at room temperature for 10 minutes.

6. Centrifuge the tube at least at 5.000 rpm for 10 minutes, open the tube and remove the supernatant carefully as much as possible.

NOTE
Don't remove the pellet.

7. Add 5 ml RNase-free Water to the tube, invert the tube three times and centrifuge at least at 5.000 rpm for 5 minutes

8. Open the tube and remove the supernatant carefully as much as possible.

NOTE
Don't remove the pellet.

9. Add 300 µl Lysis Buffer E. Resuspend the pellet completely by pipetting up and down and transfer the resuspended pellet into the 1.5 ml reaction tube containing the cleared supernatant from step 3.

10. Add 20 µl Proteinase K and 10 µl Carrier Reagent. Vortex shortly. Place the reaction tube into a thermal mixer and incubate under continuous shaking for 15 minutes at 60°C.

11. Add 450 µl Binding Buffer C and 20 µl Beads M to the lysed sample.

NOTE
Vortex the Beads M for 1 minute before use! The Binding Buffer C is viscously, please pipette carefully.

12. Mix the sample completely by vortexing for 15 seconds. Incubate the reaction tube at room temperature for 5 minutes for the binding of the nucleic acids to the magnetic particles.

13. Place the reaction tube in a magnetic rack or another magnetic particle separation equipment. Separate the beads from the supernatant and remove the supernatant as complete as possible using a pipet tip.

14. Add 500 µl Washing Buffer C and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of the beads and remove the Washing Buffer C.

15. Add 500 µl Washing Buffer C and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of beads and remove the Washing Buffer C.

16. Add 750 µl Washing Buffer D and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the Washing Buffer D.

17. Add 750 µl Washing Buffer D and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the Washing Buffer D.

NOTE
After the last washing step remove Washing Buffer D as complete as possible!

18. Place the opened reaction tube with the magnetic beads in a thermal mixer at 50 °C for 5 minutes.

NOTE
The drying step is important for all following downstream application. The ethanol must be removed completely!

19. Add 40 µl – 100 µl RNase-free water or 10 mM Tris-HCl pH 8.0-8.5, resuspend the magnetic particles completely and incubate at 50°C in a thermal mixer under continuous shaking for 5 minutes.

NOTE
The elution volume depends on expected amount of target nucleic acid

20. Perform magnetic separation of beads. Transfer the eluted DNA/RNA into a new 1.5 ml reaction tube.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted NA	
Insufficient lysis	Increase lysis time! Reduce amount of starting material. Overloading reduces yield!
Incomplete elution	Prolong the incubation time with RNase-free water or 10 mM Tris-HCl pH 8.0-8.5 to 5 minutes or repeat elution step once again. Take a higher volume of RNase-free water or 10 mM Tris-HCl pH 8.0-8.5.
Insufficient mixing with Binding Buffer C	Mix sample with Binding Buffer C by pipetting.
Low concentration of extracted NA	
Too much RNase-free water or 10 mM Tris-HCl pH 8.0-8.5was used for the elution step	Elute the NA with lower volume

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