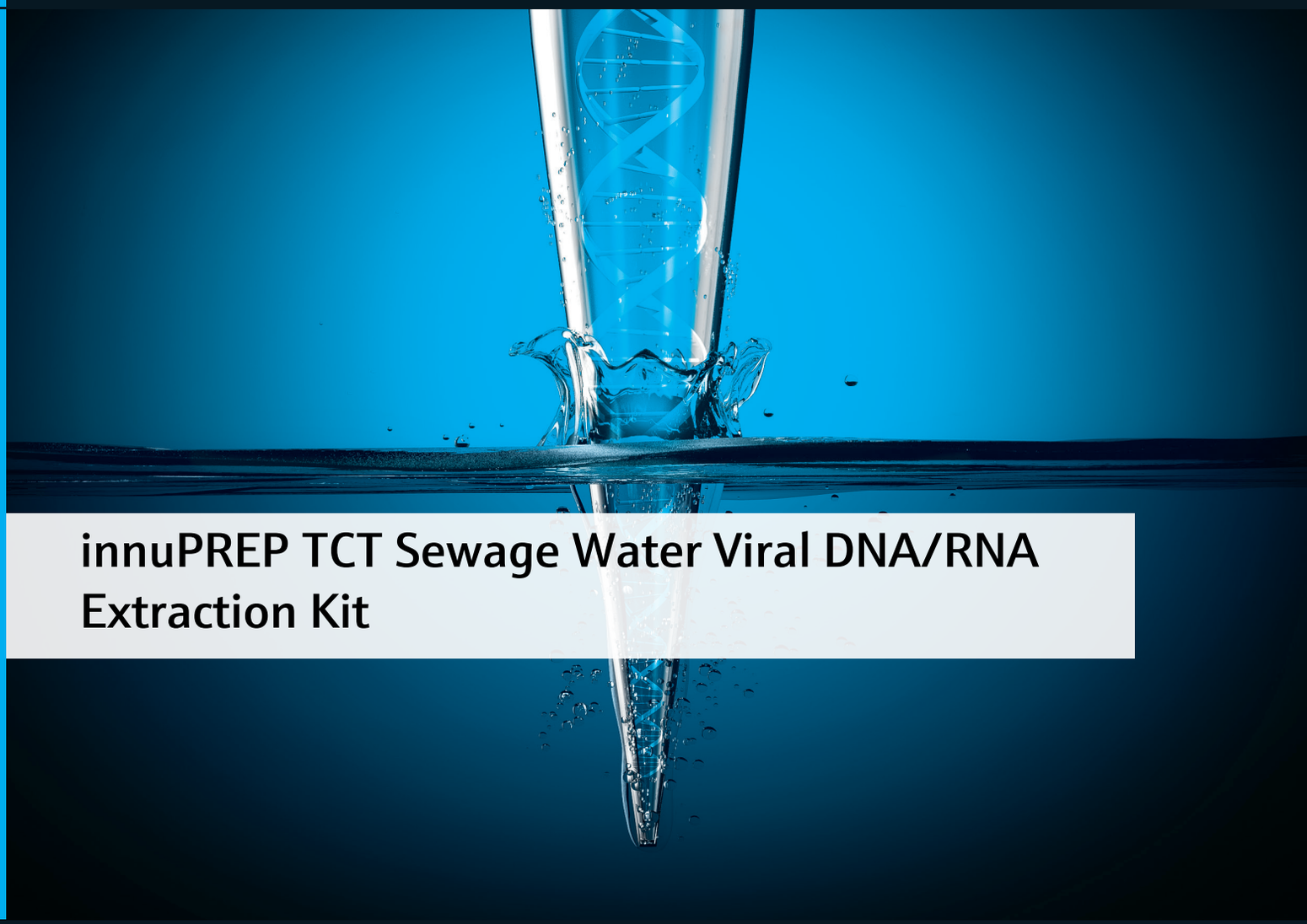
Instructions for Use
Life Science Kits & Assays



innuPREP TCT Sewage Water Viral DNA/RNA Extraction Kit

Innuscreen GmbH

Order No.:
845-TC-0010050 50 reactions
845-TC-0010010 10 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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1 Introduction

1.1 Intended use

The **innuPREP TCT Sewage Water Viral DNA/RNA Extraction Kit** has been designed especially for the extraction of DNA and RNA from viruses, bacteriophages or free circulating nucleic acids. Sewage water of different volumes (100 ml - 500 ml) can be used. The kit is based on a novel and patent-pending technology that allows biomolecules (cells, bacteria, viruses, bacteriophages, algae, free nucleic acids, proteins) contained in liquid samples to be concentrated and then made available for various other analysis methods. No filtration, ultrafiltration, ultracentrifugation or PEG precipitation is required.
In addition to using the concentrated sample for a subsequent extraction of DNA and RNA with an optimized kit, the concentrated sample can also be used for other different methods like 1. Use for microbiological cultivation methods, 2. Use of immunological methods (lateral flow test, ELISA, etc.) or 3. Cell Assays.
The innuPREP TCT Sewage Water Viral DNA/RNA Extraction Kit can be combined with kit for manual extraction of DNA/RNA as well as with different kits for automated extraction based on different automation platform like KingFisher Flex (Thermo), Felix (Analytik Jena) or Innupure C16 (Analytik Jena)

The kit is easy to handle and is divided into the following two steps:

1. Target Concentration

2. DNA/RNA extraction (manually or automated)

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully before 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 and the kit

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

Symbol	Information
REF	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> tests.
15 °C 30 °C	**Storage conditions** Store at room temperature, unless otherwise specified.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**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.
	Note / Attention Observe the notes marked in this way to avoid operating errors for obtaining correct results.

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).
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before use 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, which 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, flush eyes or skin with a large amount of water immediately.

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

ATTENTION!
Don’t eat or drink components of the kit!
The kit is designed to be handled only 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 is to be used with potential infectious human 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 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 Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240.

For more information on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.

Upon arrival, store lyophilized and dissolved **Proteinase K** and **MAG Suspension M** at 4 °C to 8 °C!

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

All other components of the **innuPREP TCT Sewage Water Viral DNA/RNA Extraction 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.

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

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product 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 **innuPREP TCT Sewage Water Viral DNA/RNA Extraction Kit** or other IST Innuscreen GmbH products,

please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com. 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 IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen 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 equivalent regulations required in other countries.

All products sold by the IST Innuscreen 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 10	Σ 50
REF	845-TC-0010010	845-TC-0010050
MAG Suspension M	2 ml	2 ml
Proteinase K	for 1 x 0.3 ml working solution	for 1 x 1.5 ml working solution
Carrier RNA	for 1 x 1.0 ml working solution	for 1 x 1.0 ml working solution
Lysis Solution RL	6 ml	30 ml
Binding Solution SBS	6 ml	30 ml
Washing Solution HS (conc.)	6 ml	30 ml
Washing Solution LS (conc.)	8 ml	36 ml
Elution Buffer	2 ml	6 ml
TCT Beads (1g)	5	25
TCT Beads (2g)	5	25
Manual	1	1

6.2 Components not included in the kit

- 1.5 ml reaction tubes
- 96–98.8 % ethanol (molecular biology grade, undenaturated)
- ddH_2O for dissolving **Proteinase K**
- RNase-free Water for dissolving **Carrier RNA** and for elution (if applicable)

7 Initial steps before starting

- Add the indicated amount of ddH_2O to each vial of **Proteinase K**, mix thoroughly and store as described above.

845-TC-0010010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-TC-0010050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of ddH_2O to each vial of **Carrier RNA**, mix thoroughly and store as described above.

845-TC-0010010	Add 1.0 ml ddH_2O to Carrier RNA.
845-TC-0010050	Add 1.0 ml ddH_2O to Carrier RNA.

- Add the indicated volume of absolute ethanol to each bottle **Washing Solution HS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-TC-0010010	Add 6 ml ethanol to 6 ml Washing Solution HS (conc.).
845-TC-0010050	Add 30 ml ethanol to 30 ml Washing Solution HS (conc.).

- Add the indicated volume of absolute ethanol to each bottle **Washing Solution LS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-TC-0010010	Add 32 ml ethanol to 8 ml Washing Solution LS (conc.).
845-TC-0010050	Add 144 ml ethanol to 36 ml Washing Solution LS (conc.).

8 Product specifications

Starting material:

- Sewage Water (100 ml – 500 ml)

9 Protocol: Target concentration

1. The initial sample with the sewage water should be allowed to stand for a sufficient time to allow the suspended solids in the sample to settle. The supernatant should be used for extraction.
2. Transfer the sample to a suitable bottle. The volume of the bottle should be about twice the volume of the sample.
3. Adding the appropriate amount of TCT Beads by combining different amounts of TCT Beads (1 g and 2 g). Shake the bottle vigorously. Incubate at room temperature until desired target volume is reached. The bottle can also be shaken briefly from time to time which speeds up the concentration somewhat.

IMPORTANT

1. The amount of TCT Beads that are used depends on the desired concentration factor (according to the desired target volume) and the time in which the concentration is to take place.
2. The shorter the concentration time should be, the more TCT Beads must be used.
3. It is also possible to perform a first concentration step and further concentrate the obtained target volume in a further concentration step.

The table below gives a few guide values that relate to initial volume, target volume and time. These are approximate values which may vary in relation to different samples.

sample volume (surface water)	**TCT Beads**	**incubation time**	**appr. volume after incubation time**
100 ml	2 g	1.5h	appr. 2 ml
100 ml	3 g	1h	appr. 5 ml
250 ml	5 g	2 h	appr. 4 ml
250 ml	10 g	30 min	appr. 10 ml
500 ml	5 g	2.5 h	appr. 100 ml
500 ml	5 g	overnight	appr. 35 ml
500 ml	6 g	overnight	appr. 15 ml

In addition, the amount of TCT Beads and the incubation time can be varied as desired and thus the volume of the concentrated sample can be specifically adjusted.

IMPORTANT
Should it happen that the entire sample has been absorbed by the beads, only a small volume of water needs to be re-added to the beads. It is then shaken briefly and the remaining volume is used.

4. After concentration has been completed, the sample is transferred to a suitable vessel and is now available for further use.

Subsequent extraction can be manual or automated and involves efficient isolation of DNA/RNA from viruses, bacteriophages or free-circulating nucleic acids.

10 Manual extraction of DNA/RNA

1. Transfer **200 µl** of concentrated sample into a 1.5 ml reaction-tube.
2. Add **300 µl Lysis Solution RL**, **20 µl Proteinase K** and **10 µl Carrier RNA**. Vortex shortly.
3. Place the reaction tube into a thermal mixer and incubate under continuous shaking for 15 minutes at 60 °C.
4. Add **450 µl Binding Solution SBS** and **15 µl MAG Suspension M** to the lysed sample.

NOTE
Vortex the MAG Suspension M for 1 minute before use! The Binding Solution SBS 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 Solution HS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of the beads and remove the Washing Solution HS.
8. Add **500 µl Washing Solution HS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of beads and remove the Washing Solution HS.
9. Add **750 µl Washing Solution LS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the Washing Solution LS.
10. Add **750 µl Washing Solution LS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the Washing Solution LS.

NOTE
After the last washing step remove Washing Solution LS 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 Elution Buffer**, 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.

11 Automated DNA/RNA extraction

Automated extraction can be performed using the KingFisher Flex device and the innuPREP AniPath DNA/RNA Kit 2.0 - KFFLX. The kit has a protocol for extracting DNA/RNA after the sample has been concentrated. Please follow the given protocol.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA/RNA	
Insufficient lysis	Increase lysis time. Reduce amount of starting material.
Incomplete elution	Prolong the incubation time with RNase-free Water or Elution Buffer. Take a higher volume of RNase-free Water or Elution Buffer.
Low concentration of extracted viral DNA/RNA	
Too much RNase-free water or Elution Buffer	Elute the DNA/RNA in a lower volume of RNase-free Water or Elution Buffer.

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