

Instructions for Use

Life Science Kits & Assays



PME Sewage Water Enrichment Tool

Order No.:

845-IR-0011050 50 reactions

Publication No.: HB_IR-0011_e_230215

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 **PME Sewage Water Enrichment Tool** has been designed for the enrichment of viruses, bacteriophages and free-circulating DNA/RNA (e.g. plasmid associated DNA coding for bacterial resistances) the isolation of DNA and RNA from sewage water samples.

The dissolved biomolecule/polymer complex (200 µl) can be transferred and processed according the 200 µl cell-free body fluids protocol to the reagent plastic of the following kits:

innuPREP Anipath DNA/RNA Kit-IPC16 (845-IPP/PPP/IPS-8016016/96/480) followed by extraction on the InnuPure C16touch

or

innuPREP Anipath DNA/RNA Kit-KFFLX (845-KF5296096/480/9600) followed by extraction on the KingFisher Flex 96

or

innuPREP Anipath DNA/RNA Kit-FX (845-FX-2396096/480) followed by extraction on the CyBio Felix.

The extraction process starts with 20 ml of sewage water sample. After sedimentation of solid material 10 ml of the resulting supernatant will be used for the extraction of DNA and RNA.

The kit is intended for use by professional users. The kit has been designed to be used for a wide range of different downstream applications, like amplification reactions and further analytical procedures.



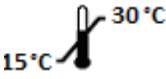







CONSULT INSTRUCTION FOR USE

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 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 and the kit“ p. 3).
- Work steps are numbered.

2 Safety precautions

NOTE

Read through this chapter carefully before 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 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

All kit components are shipped at ambient temperature.

Store **Enrichment Reagent VCR-1** at 4 °C to 8 °C.

All other components of the **PME Sewage Water Enrichment Tool** 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.

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 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 innuPREP Sewage Water Enrichment Tool 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. 7). 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 equivalents in other countries.


All products sold by 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 Components included in the kit

 50	
REF	845-IR-0011050
Enrichment Reagent VCR-1	5 x 1.2 ml
Enrichment Reagent VCR-2	5 ml
PBS, 1 x	15 ml
Manual	1

6.2 Components not included in the kit

- 50 ml tubes
- RNase-free Water for washing step

7 Product specifications

1. Starting material:
 - 20 ml sewage water
2. Time for isolation:
 - Approximately 30 minutes including all steps

8 Initial steps before starting

- Centrifugation steps should be carried out at room temperature.

9 Protocol: Enrichment of viruses, phages or free circulating DNA/RNA from sewage supernatant

9.1 Preliminary steps

1. Transfer 20 ml of sewage water sample into a 50 ml tube. Centrifuge at 2.500 x g for 10 minutes.
2. Transfer 10 ml clear supernatant into a new 50 ml tube.

9.2 Isolation of DNA/RNA from supernatant

1. Add 100 µl Enrichment Reagent VCR-1. Shake the tube shortly.
2. Add 100 µl Enrichment Reagent VCR-2. Shake the tube shortly. Incubate at room temperature for 10 minutes.
3. Centrifuge the tube at least at 2.500 x g 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 2.500 x g for 5 minutes.
5. Open the tube and remove the supernatant carefully as much as possible.

NOTE

Don't remove the pellet.

6. Add 200 µl 1 x PBS and resuspend the pellet completely by pipetting up and down.
5. Perform further extractions with the dissolved biomolecule/polymer complex (200 µl) which can be transferred to the reagent plastic of each special kit (→ "Introduction" p. 2) and processed according the 200 µl cell-free body fluids protocol.

10 Troubleshooting

Problem / probable cause	Comments and suggestions
No pellet after first centrifugation step	
Insufficient addition of Enrichment Reagent VCR-1 or VCR-2	<p>Make sure that both Enrichment Reagent VCR-1 and VCR-2 are added to the reaction tube.</p> <p>Make sure that the right volume of VCR-1 and VCR-2 are added.</p>
Insufficient centrifugation	<p>Make sure that centrifugation steps are carried out as describe in the manual.</p> <p>Otherwise repeat centrifugation.</p>
Discarding of pellet	<p>Ensure that the pellet is not discarded during removing the supernatant.</p> <p>In some cases, the pellet cannot be seen until the supernatant is removed completely.</p>
Pellet is difficult to dissolve	
Too much addition of Enrichment Reagent VCR-1 or VCR-2	<p>Make sure that both Enrichment Reagent VCR-1 and VCR-2 are added as described in protocol.</p>
Not enough of 1 x PBS added to pellet	<p>We recommend adding minimum 200 µl of 1 x PBS. Depending on the sample properties adding higher volumes of 1 x PBS might be necessary.</p>
Pipette tip is clogged while dissolving the pellet	<p>Cut the slide edge of pipette tip and try to transfer the pellet as much as possible.</p>

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