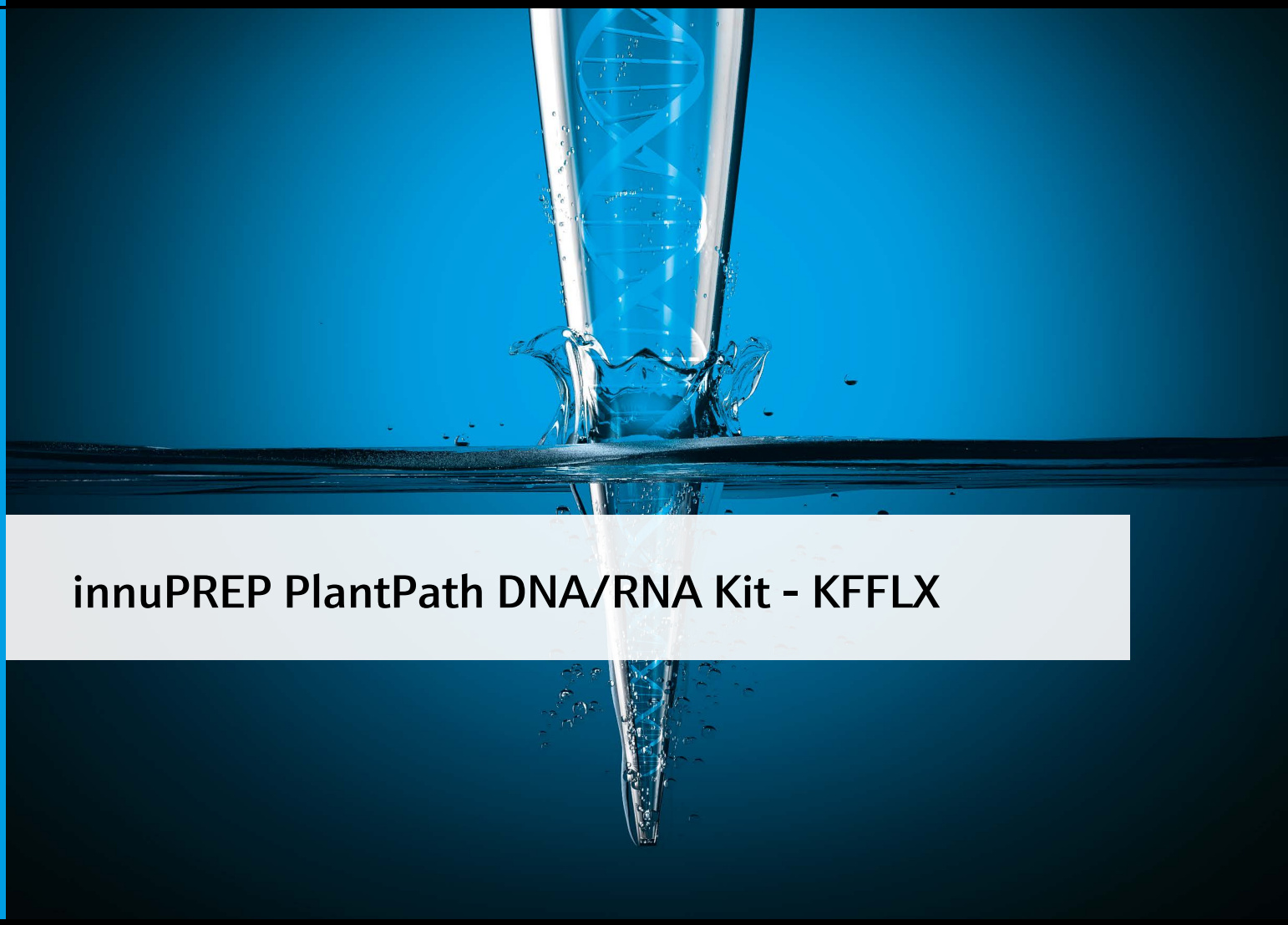
Instructions for Use
Life Science Kits & Assays



innuPREP PlantPath DNA/RNA Kit - KFFLX

Innuscreen
innovative
Sensor
Technology

Order No.:

845-KF-5696096	96 reactions
845-KF-5696480	480 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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Contents

1 Introduction

1.1 Intended use

The **innuPREP PlantPath DNA/RNA Kit - KFFLX** has been designed for automated isolation of viral or bacterial DNA and RNA from different kinds of starting material on the KingFisher FLEX. The extraction procedure is based on a new kind of chemistry. The kit has been successfully tested for:

Clavibacter sepedonicus (Cs)

Ralstonia solanacearum (Rs)

Viral RNA out of different kinds of potato viruses

The extraction procedure takes place on the magnetic particle processor KingFisher FLEX and allows the parallel extraction of up to 96 samples. The kit contains a Carrier Mix. The Carrier Mix consists of a necessary Carrier RNA as well as a synthetic DNA fragment and MS2 RNA. Both can be used as internal extraction controls. The proof can be provided by means of available assays from IST Innuscreen GmbH. In addition, individual internal controls can be used. No data are available on the rate of recovery of individual used internal controls. There can be no guarantee for the recovery of individual internal controls. Please note that the use of individual controls based on MS2 RNA sequences may lead to higher detection signals with the MS2 RNA from the Carrier Mix.

It is important to note, that the kit should be used with an internal extraction control and corresponding detection assays to monitor the purification, amplification, and detection processes.

Please note that the eluates contain Carrier Mix.

The detection limit for certain viruses depends on the individual procedures, for example in-house PCR or commercial used detection

assays. We can give no warranty for the efficiency of extraction for different kinds of viruses.

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. Diagnostic results generated using the extraction procedure in conjunction with diagnostic tests should be interpreted regarding other clinical or laboratory results. To reduce irregularities in diagnostic results, internal controls for downstream applications should be used.

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 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> reactions.
15 °C 30 °C	**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	**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 ensure correct function of the kit and 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. 4).
- 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 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 regulations.

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 General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.

- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four hours or more before use. Autoclaving will not inactivate RNase activity completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C followed by autoclaving or heating to 100 °C for 15 minutes to remove residual DEPC.
- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5 % SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free water.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

4 Storage conditions

All kit components are shipped at ambient temperature.

Store lyophilized and dissolved **Proteinase K and MAG Suspension F**

at 4 °C to 8 °C.

Store lyophilized and dissolved **Carrier Mix and Enzyme A and Storage Buffer A** at -22 °C to -18 °C.

All other components of the innuPREP PlantPath DNA/RNA Kit - KFFLX 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 solve these precipitates by careful warming. Before every use make sure that all components have room temperature.

5 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 PlantPath DNA/RNA Kit - KFFLX 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.com. For other countries please contact your local distributor.

6 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. 11). 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

This kit is for research use only!

7 Kit components

7.1 Components included in the kit

	Σ 96	Σ 480
REF	845-KF-5696096	845-KF-5696480
MAG Suspension F	1.1 ml	5 x 1.1 ml
Lysis Solution MA	25 ml	120 ml
Binding Solution V	60 ml	250 ml
Enzyme A (Lysozyme)	20 mg - for 2 ml working solution	100 mg - for 10 ml working solution
Storage Buffer A	2 ml	10 ml
Proteinase K	for 2 x 1.5 ml working solution	for 8 x 1.5 ml working solution
Carrier Mix	For 2 x 1.25 ml working solution	For 6 x 1.25 ml working solution
Washing Solution A	180 ml	2 x 600 ml
Washing Solution B2 (conc.)	80 ml	2 x 240 ml
RNase-free Water	15 ml	2 x 30 ml
Manual	1	1

7.2 Components not included in the kit

- 50 mM phosphate buffer
- 96 %–99.8 % ethanol (molecular biology grade, undenatured)
- ddH_2O; ultrapure for dissolving Proteinase K
- 96-well-plates and tip combs for KingFisher Flex

8 Usage of Carrier Mix

Besides carrier RNA, the **Carrier Mix** contains an Internal Control DNA and Internal Control RNA (IC DNA and IC RNA). Both can be detected by real-time PCR using the corresponding assay.

Name	Amount	Order No.
innuDETECT Internal Control DNA/RNA Assay	100 rxn	845-ID-0008100

If customized extraction controls are used, please add these components to the mixture of **Lysis Solution MA / Carrier Mix** (→ "Initial steps before starting" p. 12).

9 Product specifications

1. Starting material:

- 200µl potato homogenate

2. Time for isolation:

- 55 minutes – automated extraction

10 Initial steps before starting

- Add 1.5 ml ddH_2O to each vial of lyophilized **Proteinase K**, mix thoroughly and store as described above.
- Add the indicated amount of absolute ethanol to **Washing Solution B2 (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-KF-5696096	Add 120 ml ethanol to 80 ml Washing Solution B2 (conc.)
845-KF-5696480	Add 360 ml ethanol to 240 ml Washing Solution B2 (conc.)

- Add 1.25 ml RNase-free Water to each vial of **Carrier Mix**, mix thoroughly and store as described above.
- Add the entire volume of **Storage Buffer A** to the vial of **Enzyme A** and mix thoroughly. Always keep the vial firmly closed!

845-KF-5696096	Add 2 ml Storage Buffer A to 20 mg Enzyme A
845-KF-5696480	Add 10 ml Storage Buffer A to 100 mg Enzyme A

- Prepare **Lysis Master Mix** according to the special protocols.

Component	1 sample	48 samples	96 samples
Lysis Solution MA	200 µl	10 ml	20 ml
Carrier Mix	10 µl	0.5 ml	1.0 ml
Proteinase K	20 µl	1.0 ml	2.0 ml
Lysozym (10 mg/ml)	20 µl	1.0 ml	2.0 ml
Final volume	**250 µl**	**12.5 ml**	**25.0 ml**

- Avoid freezing and thawing of starting material.

11 Sample Preparation

11.1 Protocol: Isolation from potatoes

1. Label one Deep Well Plate with “Lysis Plate”.
2. 25 navels (potato) are homogenized in 5 mL of 50 mM phosphate buffer.
3. Transfer **200 µl** of the homogenate into the wells of the Deep Well Plate labeled with “Lysis Plate”.
4. Transfer **250 µl Lysis Master Mix** to each well used.

12 Automated Extraction

12.1 Prefilling of Deep Well Plates

Label and fill the Deep Well plates according to the table below.

Plate	Label	Content
Deep Well	Lysis Plate	200 µl sample + 250 µl Lysis Master Mix
Deep Well	Washing A	900 µl Washing Solution A
Deep Well	Washing A	900 µl Washing Solution A
Deep Well	Washing B2	900 µl Washing Solution B2
Deep Well	Washing B2	1000 µl Washing Solution B2
96 Plate	Elution Plate	100 µl RNase-free Water
Deep Well	Tip Comb Plate	96 Well Tip Comb

12.2 Loading Deep Well Plates to KingFisher FLEX

1. Turn on and select the protocol “PlantPath_KFFLX” on KingFisher FLEX instrument and start the run.
2. Follow the instruction and load prefilled Deep Well Plates successive to the sample tray:

   Tip Comb Plate

   Elution Plate

   Washing Solution A

   Washing Solution A

   Washing Solution B2

   Washing Solution B2

   Lysis Plate (Sample and Lysis Master Mix)

12.3 Starting the automated extraction

1. The automated extraction process starts with sample lysis. After sample lysis the automated run stops.
2. After the device has stopped, take the “Lysis Plate” out of the device and add **10 µl** of well mixed **MAG Suspension F** and **490 µ**l of **Binding Solution V** to the lysed samples.

NOTE

Mix the **MAG Solution F** well by vortexing for 1 minute.

3. After addition of **MAG Suspension F** and **Binding Solution V** place the “Lysis Plate” back to the KingFisher Flex and continue the extraction process by starting the KingFisher Flex again (you will find the instruction on the display of the KingFisher Flex).

IMPORTANT NOTE

After finishing the extraction protocol, the Elution Plate contains the isolated DNA/RNA. Store the DNA/RNA under adequate conditions. We recommend storing the extracted RNA at-82 to −78°C.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Poor lysis of starting material	
Insufficient disruption or homogenization	After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no total RNA eluted	
Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!
DNA contamination	
Too much starting material	Reduce amount of starting material.
Incorrect lysis of starting material	Use the recommended techniques for lysis of cell pellet.
Total RNA degraded	
RNA source inappropriately handled or stored	Ensure that the starting material is fresh! Ensure that the protocol, especially the first steps, has been performed quickly.
RNase contamination of solutions; Receiver Tubes, etc.	Use sterile, RNase-free filter tips. Before every preparation clean up the pipette, the devices and the working place. Always wear gloves!
Total RNA does not perform well in downstream applications (e.g. RT-PCR)	
Salt carryover during elution	Ensure that **Washing Solution A** and **Washing Solution B2** are at room temperature. Checkup Washing Solution for salt precipitates. If there are any precipitate dissolves these precipitate by carefully warming.
Carryover of magnetic beads	
Eluate contains carryover of magnetic particles	Place the plate on a magnet or centrifuge at maximum speed for 3 minutes. Then, pipet the supernatant with DNA/RNA into a new plate.

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