Instructions for Use
Life Science Kits & Assays

innuPREP AniPath DNA/RNA Kit – KFFLX

analytikjena
An Endress+Hauser Company

Order No.:

845-KF-5296096 96 reactions
845-KF-5296480 480 reactions
845-KF-5296960 960 reactions

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1 Introduction

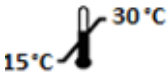
1.1 Intended use

The innuPREP AniPath 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:

Canine Distemper Virus
Bluetongue Virus
Bovine Viral Diarrhoea Virus
Porcine Circovirus 2
Koi Herpesvirus
Porcine Reproductive and Respiratory Syndrome Virus
Staphylococcus aureus
E. coli virulence factors
Chlamydia spp.
Leptospira interrogans
Lawsonia intracellularis
Mycobacterium avium paratuberculosis
Toxoplasma gondii

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.
	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. 3).
- Working 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, 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 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 please download the Safety Data Sheet (SDS) from our website (www.analytik-jena.com). Find the SDS of each product and its components in the “Downloads”-folder.

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. Upon arrival, store lyophilized **Proteinase K** at 4 °C to 8 °C. Divide dissolved Proteinase K into aliquots and store at -22 °C to -18 °C. Repeated freezing and thawing will reduce the activity dramatically!

Store lyophilized **Carrier Mix** at -22 °C to -18 °C. Divide dissolved **Carrier Mix** into aliquots and store at -22 °C to -18 °C. Do not freeze and thaw the **Carrier Mix** more than 3 times.

Store **MAG Suspension** at 4 °C to 8 °C.

The mixture of **Lysis Solution V** and **Carrier Mix** is stable for a maximum of 7 days if stored at 4 °C to 8 °C.

All other components of the innuPREP AniPath 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 Analytik Jena AG 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 AniPath DNA/RNA Kit - KFFLX or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. 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. 9). Since the performance characteristics of Analytik Jena AG kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits using other protocols than those described below. Analytik Jena AG 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 Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE
For research use only!

7 Kit components

7.1 Components included in the kit

	Σ 96	Σ 480	Σ 960
REF	845-KF-5296096	845-KF-5296480	845-KF-5296960
MAG Suspension F	1.1 ml	5 x 1.1 ml	10 x 1.1 ml
Lysis Solution V	30 ml	140 ml	1 x 250 ml
Binding Solution V	2 x 40 ml	2 x 160 ml	1 x 650 ml
Proteinase K	for 2 x 1.5 ml working solution	for 8 x 1.5 ml working solution	for 16 x 1.5 ml working solution
Carrier Mix	For 2 x 1.25 ml working solution	For 6 x 1.25 ml working solution	For 12 x 1.25 ml working solution
Washing Solution HS (conc.)	30 ml	2 x 75 ml	350 ml
Washing Solution LS (conc.)	40 ml	3 x 40 ml	2 x 130 ml
RNase-free Water	15 ml	2 x 30 ml	4 x 30 ml
Manual	1	1	1

7.2 Components not included in the kit

- 1.5 ml tubes
- 96 %–99.8 % ethanol (molecular biology grade, undenaturated)
- PBS optional (for isolation of viral RNA from stool sample)
- physiological saline (optional)
- 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 V / Carrier Mix** (→ "Initial steps before starting" p. 10).

9 Product specifications

1. Starting material:

- Cell-free body fluids and cell culture supernatant (e.g. serum, plasma, cerebrospinal fluid)
- Whole blood samples (200 µl)
- Swabs from nasopharyngeal samples (e.g. Influenza testing)
- Tissue samples (up to 10 mg)
- Stool samples (50–100 mg)

2. Time for isolation:

- Approximately 60 minutes

10 Initial steps before starting

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

845-KF-5296016	Add 30 ml ethanol to 30 ml Washing Solution HS (conc.)
845-KF-5296096	Add 75 ml ethanol to 75 ml Washing Solution HS (conc.)
845-KF-5296480	Add 350 ml ethanol to 350 ml Washing Solution HS (conc.)

- Add to **Washing Solution LS (conc.)** the indicated amount of absolute ethanol and mix thoroughly. Keep the bottle always firmly closed!

845-KF-5296016	Add 160 ml ethanol to 40 ml Washing Solution LS (conc.)
845-KF-5296096	Add 160 ml ethanol to 40 ml Washing Solution LS (conc.)
845-KF-5296480	Add 520 ml ethanol to 130 ml Washing Solution LS (conc.)

- Add to each vial of **Carrier Mix** 1.25 ml RNase-free Water, mix thoroughly and store as described above.
- Prepare **Lysis Solution V / Carrier Mix** according to the table below and store as described above.

Component	8 samples	48 samples	96 samples	n samples
Lysis Solution V	2 ml	12 ml	24 ml	250 µl x n samples
Carrier Mix	0.1 ml	0.6 ml	1.2 ml	12.5 µl x n samples
Final volume	2.1 ml	12.6 ml	25.2 ml	262.5 µl x n samples

NOTE

The preparation of **Carrier Mix / Lysis Solution** mixture is not necessary for all sample types. Refer to the specific protocol used.

- Avoid freezing and thawing of starting material.
- Label a Deep Well Plate with “Lysis Plate”.

11 Protocols for isolation of viral nucleic acids

11.1 Protocol 1: Isolation from cell-free body fluids and cell culture supernatants

NOTE

Using cell free body fluids we recommend the addition of Carrier Mix. The **Carrier Mix** is added to the **Lysis Solution V** as described under “Initial steps before starting” on p. 10.

1. Transfer **200 µl Lysis Solution V / Carrier Mix** into the wells of the Deep Well Plate labeled with “Lysis Plate”.
2. Add **200 µl** of the sample to the Deep Well Plate containing **Lysis Solution V / Carrier Mix**.
3. Add **20 µl Proteinase K** to each well used.

11.2 Protocol 2: Isolation from 200 µl whole blood samples

1. Transfer **200 µl Lysis Solution V** into the wells of the Deep Well Plate labeled with “Lysis Plate”.
2. Add **200 µl** of the whole blood sample to the Deep Well Plate containing **Lysis Solution V**.
3. Add **20 µl Proteinase K** to each well used.

NOTE

If the volume of the blood sample is less than 200 µl adjust with PBS to 200 µl.

11.3 Protocol 3: Isolation from nasopharyngal samples (e.g. for Influenza testing)

NOTE

Using cell free body fluids we recommend the addition of Carrier Mix. The **Carrier Mix** is added to the **Lysis Solution V** as described under "Initial steps before starting" on p. 10.

1. Transfer **200 µl Lysis Solution V / Carrier Mix** into the wells of the Deep Well Plate labeled with "Lysis Plate".
2. Place the swabs into tubes containing physiological saline (0.9 % NaCl) or PBS and incubate under continuously shaking for 20 minutes.
3. Squeeze out the swab and remove the swab.
4. Add **200 µl** of the liquid sample into the DW Plate containing **Lysis Solution V/Carrier Mix**.
5. Add **20 µl Proteinase K** to each well used.

11.4 Protocol 4: Isolation from tissue homogenates

NOTE

Co-extraction of genomic nucleic acids can inhibit downstream PCR or Real-time PCR applications!

1. Homogenize the tissue samples using bead based homogenizers (e.g. SpeedMill Anaslytik Jena AG). For optimized results use 5-10 mg of tissue sample.
2. Transfer the tissue sample into a homogenization tube and add **400 µl** ddH_20 (RNase free) or PBS.
3. After homogenization centrifuge the sample at 10,000 x g for 2 minutes.
4. Transfer **200 µl Lysis Solution V** into the wells of the Deep Well Plate labeled with "Lysis Plate".

5. Add **200 µl** of the homogenized tissue sample to the Deep Well Plate containing **Lysis Solution V**.
6. Add **20 µl Proteinase K** to each well used.

11.5 Protocol 5: Isolation from stool samples (e.g. from Norovirus)

NOTE

In some cases the initial fecal sample is mixed with special ELISA Buffer for subsequent ELISA detection of different viruses. In this case use Option 2.

Option 1: Standard procedure

1. Transfer about 50–100 mg of stool sample into a 1.5 ml reaction tube.
2. Add **250 µl PBS** (not included in the kit). Vortex the tube for 10 seconds.
3. Centrifuge the tube at maximum speed for 3 minutes.
4. Transfer **200 µl Lysis Solution V** into the Deep Well Plate labeled with “Lysis Plate”.
5. Add **200 µl** of the clear supernatant to the Deep Well Plate containing Lysis Solution V.
6. Add **20 µl Proteinase K** to each well used.

Option 2: Fecal sample is already mixed with ELISA Buffer

1. Use **250** µl of the sample, transfer it into a 1.5 ml reaction tube and centrifuge the tube at maximum speed for 3 minutes.
2. Transfer **200 µl Lysis Solution V** into the Deep Well Plate labeled with “Lysis Plate”.
3. Add **200 µl** of the clear supernatant to the Deep Well Plate containing Lysis Solution V.
4. Add **20 µl Proteinase K** to each well used.

12 Automated extraction using KingFisher FLEX

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	Lysed samples (including Lysis Solution V / Carrier Mix and Proteinase K)
Deep Well	Washing Plate 1	600 µl Washing Solution HS
Deep Well	Washing Plate 2	600 µl Washing Solution LS
Deep Well	Washing Plate 3	600 µl Washing Solution LS
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 “INNU_Viral_DNA_RNA_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 Plate 3

   Washing Plate 2

   Washing Plate 1

   Lysis Plate (containing sample, Lysis Solution V and Proteinase K)

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 **600 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 start 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 −80°C.

If the eluate contains carryover of magnetic particles, place the plate on a magnet or centrifuge the plate at maximum speed for 3 minutes. Pipet the supernatant with DNA/RNA into a new plate.

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 HS** and **Washing Solution LS** are at room temperature. Checkup Washing Solution for salt precipitates. If there are any precipitate dissolves these precipitate by carefully warming.

engl. 03 / 20 – Analytik Jena AG, Jena

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