Instructions for Use
Life Science Kits & Assays

innuPREP SE HMW DNA Kit - KFFLX

Innuscreen GmbH

Order No.:
845-KF-4296096 96 reactions
845-KF-4296480 480 reactions

Publication No.: HB_KF-4296_e_220829

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

Manufacturer and Distributor:
IST Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin · Germany
Made in Germany!
Phone +49 30 9489 3380
Fax +49 30 9489 3381
info.innu@ist-ag.com

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1 Introduction

1.1 Intended use

The **innuPREP SE HMW DNA Kit - KFFLX** has been designed for automated isolation of high molecular weight DNA (HMW) from bacteria, yeasts, tissue samples and rodent tails.

The kit is based on the patented SmartExtraction Technology using Smart Modified Surfaces invented by IST Innuscreen GmbH.

The extraction process is based on adsorption of the genomic DNA to Smart Modified Surfaces and it needs no magnetic particles for DNA binding. That means, the DNA binds direct on the surface of the modified KingFisher Flex Tip Combs. After washing, the genomic DNA is eluted from the Smart Modified Surfaces and is ready for use in subsequent downstream applications.

The whole extraction process just needs simple mixing up and down of the modified Tip Combs. The process is very fast and gives no limitation regarding the binding capacity. So, the kit is optimized to get a maximum of yield and quality.

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> 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 and the kit“ p. 3).
- Working 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, 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 and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 Storage conditions

All kit components are shipped at ambient temperature.

Upon arrival, store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C and **RNase A** at -22 bis −18°C.

All other components of **the innuPREP SE HMW DNA 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.

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 SE HMW DNA 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-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. 9). 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

	Σ 96	Σ 480
REF	845-KF-4296096	845-KF-4296480
Lysis Solution CBV	50 ml	250 ml
Proteinase K	for 2 x 1.5 ml working solution	for 10 x 1.5 ml working solution
RNase A	500 µl	4 x 500 µl
Binding Optimizer	5 ml	2 x 15 ml
Washing Solution LS (conc.)	45 ml	2 x 130 ml
Washing Solution ER	85 ml	450 ml
RNase free Water	25 ml	3 x 30 ml
KF96 Tip Comb with DW Plate	1	5
KF96 modified Tip Comb with DW Plate	1	5
KF96 DW Plate	6	30
Manual	1	1

6.2 Components not included in the kit

- PBS, 1x
- 96 %–99.8 % ethanol (molecular biology grade, undenaturated)
- 80 % ethanol for washing plate 3/4
- Isopropanol
- ddH_2O; ultrapure for dissolving Proteinase K

6.3 Components needed for isolation from bacteria

- Lysozyme (stock solution 10 mg/mL (400 U/µL))
- Mutanolysin (stock solution 0.4 U/µL)
- Lysostaphin (stock solution 0.4 U/µL)
- TE-Buffer

Alternatively:

- innuPREP Bacteria Lysis Booster (845-KA-1000050, 50 rxn, IST Innuscreen GmbH)

6.4 Components needed for isolation from yeasts

- Yeast Digest Buffer (50 mM KH_2PO_4, 10 mM DTT, pH 7.5)
- Lyticase (stock solution 10 U/µL)

7 Product specifications

1. Starting material:

- Tissue samples (1 mg – 20 mg)
- Rodent tails (0.1-0.2 cm)
- Bacteria / Yeast cell pellets (1 x 10^8 – 1 x 10^{12} cells)

2. Time for automated extraction:

- Protocol for bacteria and yeast

  SE_DNA_2 →55 minutes (excluding cell wall pre-lysis)
- Protocol for tissue and rodent tails

  SE_DNA_3 →122 minutes (including lysis of the tissue / rodent tail)

8 Initial steps before starting

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

845-KF-4096096	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-KF-4096480	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of ethanol to **Washing Solution LS (conc.)** and mix thoroughly. Always keep the bottles firmly closed!

845-KF-4096096	Add 180 ml ethanol to 45 ml **Washing Solution LS**.
845-KF-4096480	Add 520 ml ethanol to 130 ml **Washing Solution LS**.

9 Sample preparation

9.1 Sample preparation of tissue samples and rodent tails

1. Cut the sample into small pieces and transfer them into a well of the KF96 DW Plate (labeled with “Lysis Plate”).
2. Add **400 µl Lysis Solution CBV** and **30 µl Proteinase K**.

NOTE
Optionally add to each sample 5 µl RNase A (10mg/ml).

3. Proceed with “Automated extraction using KingFisher Flex” on p.14.

9.2 Sample preparation of bacteria cell pellets

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 min at 3,000 x g) and discard the supernatant.
2. Resuspend the bacterial cell pellet in 170 µL TE Buffer

9.2.1 Pre-lysis of cell walls using single enzymes

Gram-negative bacteria

Although gram-negative bacteria do not require a pre-lysis step, using Lysozyme (not included in the kit) can enhance the efficiency of lysis.

Using Lysozyme: stock solution of Lysozyme: 10 mg/mL (400 U/µL)

1. Add 20 µL Lysozyme to the resuspended cells and incubate at 37 °C for 30 min under continuous shaking.
2. Transfer the sample into a well of the KF96 DW Plate (labeled with “Lysis Plate”).
3. Add **200 µl Lysis Solution CBV** and **30 µl Proteinase K**.

NOTE
Optionally add to each sample 5 µl RNase A (10mg/ml).

4. Proceed with “Automated extraction using KingFisher Flex” on p.14.

Staphylococcus spp.

For lysis of *Staphylococcus* the enzyme Lysostaphin is recommended (not included in the kit).

Using Lysostaphin: stock solution of Lysostaphin: 0.4 U/µL

1. Add 10 µL Lysostaphin to the resuspended cells and incubate at 37 °C for 30 min under continuous shaking.
2. Add **200 µl Lysis Solution CBV** and **30 µl Proteinase K**.

NOTE
Optionally add to each sample 5 µl RNase A (10mg/ml).

3. Proceed with "Automated extraction using KingFisher Flex" on p.14.

Gram-positive bacteria

Gram-positive bacteria require a pre-lysis step using Mutanolysin and/or Lysozyme (not included in the kit) Lysozyme and Mutanolysin exert synergistic activity. Using both enzymes together will increase the yield of isolated nucleic acids.

Using Lysozyme: stock solution of Lysozyme: 10 mg/mL (400 U/µL)

1. Add 20 µL Lysozyme to the resuspended cells and incubate at 37 °C for 30 min under continuous shaking.

Using Mutanolysin: stock solution of Mutanolysin: 0.4 U/µL

2. Add 5 µL Mutanolysin to the resuspended cells and incubate at 37 °C for 30 min under continuous shaking.
3. Add **200 µl Lysis Solution CBV** and **30 µl Proteinase K**.

NOTE
Optionally add to each sample 5 µl RNase A (10mg/ml).

4. Proceed with "Automated extraction using KingFisher Flex" on p.14.

9.2.2 Pre-lysis of cell walls using innuPREP Bacteria Lysis Booster

The innuPREP Bacteria Lysis Booster Kit has been developed for a highly efficient pre-lysis of bacterial cell walls thus generating spheroplasts. This new mixture of different enzymes boosts the lysis of all bacteria in particular hard-to-lyse microorganisms like *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Bacillus* and *Clostridium*.

1. Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.
2. Add **20 µL of the prepared enzyme mix** to the sample and vortex it shortly. Incubate the sample for 30 min at 37 °C.
3. Add **200 µl Lysis Solution CBV** and **30 µl Proteinase K**.

NOTE
Optionally add to each sample 5 µl RNase A (10mg/ml).

4. Proceed with "Automated extraction using KingFisher Flex" on p.14.

9.3 Sample preparation of yeast cells

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 min with 3,000 x g) and discard the supernatant.
2. Resuspend the yeast cell pellet in 200 µL Yeast Digest Buffer (→ see "Components needed for isolation from yeasts", p. 8).

9.3.1 Pre-Lysis of cell walls

Using Lyticase: stock solution of Lyticase is 10 U/µL

1. Add 10 µL Lyticase to the resuspended cells and incubate at 37 °C for 30min under continuous shaking.
2. Transfer the sample into a well of the KF96 DW Plate (labeled with "Lysis Plate").
3. Add **200 µl Lysis Solution CBV** and **30 µl Proteinase K**.

NOTE
Optionally add to each sample 5 µl RNase A (10mg/ml).

4. Proceed with "Automated extraction using KingFisher Flex" on p.14.

10 Automated extraction using KingFisher Flex

10.1 Prefilling of Deep Well Plates

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

Plate	Label	Content
Deep Well	Lysis Plate	CBV, Proteinase K, RNase and tissue / rodent tail sample or pre-lysed bacteria / yeast sample described as before
Deep Well	Washing Plate 1	1000 µl Washing Solution LS
Deep Well	Washing Plate 2	1000 µl Washing Solution LS
Deep Well	Washing Plate 3	1000 µl 80% Ethanol
Deep Well	Washing Plate 4	800 µl Washing Solution ER
Deep Well	Elution Plate	200 µl RNase-free Water
Deep Well	Modified Tip Comb Plate	96 Well Tip Comb modified
Deep Well	Tip Comb Plate	96 Well Tip Comb

10.2 Loading Deep Well Plates to KingFisher Flex

1. Turn on and select the protocol

   “SE_DNA_2“ for bacteria or yeasts or

   “SE_DNA_3“ for tissue/rodent tail samples

   on KingFisher FLEX instrument and start the run.

2. Follow the instruction and load prefilled Deep Well Plates and Tip Combs successive to the sample tray.

10.3 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.

3. Add **50 µl Binding Optimizer** and **400 µl Isopropanol** to each sample.

4. After addition of **Binding Optimizer** and **Isopropanol** 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).

5. After finishing the extraction protocol, the Elution Plate contains the isolated HMW DNA.

IMPORTANT NOTE HIGH MOLECULAR WEIGHT DNA
The HMW DNA might be very viscous. The dissolving step is crucial for successful extraction and for a maximum of yield. If the DNA content is too high, increase the amount of Elution Buffer.

HMW gDNA needs time to relax. It is generally not recommended to work with freshly eluted DNA unless significant effort is made to ensure even DNA resuspension. Letting a sample relax overnight or for several days facilitates homogenization. If possible, it is recommended that HMW DNA is extracted several days or a week prior to being needed for downstream application.

If you do not need high molecular weight DNA you can shear the DNA e.g. by using ultrasound or by passing the eluate through a needle or a shredder spin filter unit.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Preparation without Binding Optimizer and Isopropanol	Pay special attention that Binding Optimizer and Isopropanol was added to the lysed sample!
High viscosity of extracted DNA / Inhomogeneous DNA solution	
Insufficient amount of Elution Buffer	Refer to the note of HMW DNA and the DNA with a higher volume of RNase-free Water
Relax time to short	Refer to the note of HMW DNA and let the DNA relax overnight at 2-8°C
Degraded or sheared DNA	
Old material insufficient	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion

IST Innuscreen GmbH
Robert-Rössle-Str.10
13125 Berlin · Germany

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

IST Innuscreen GmbH