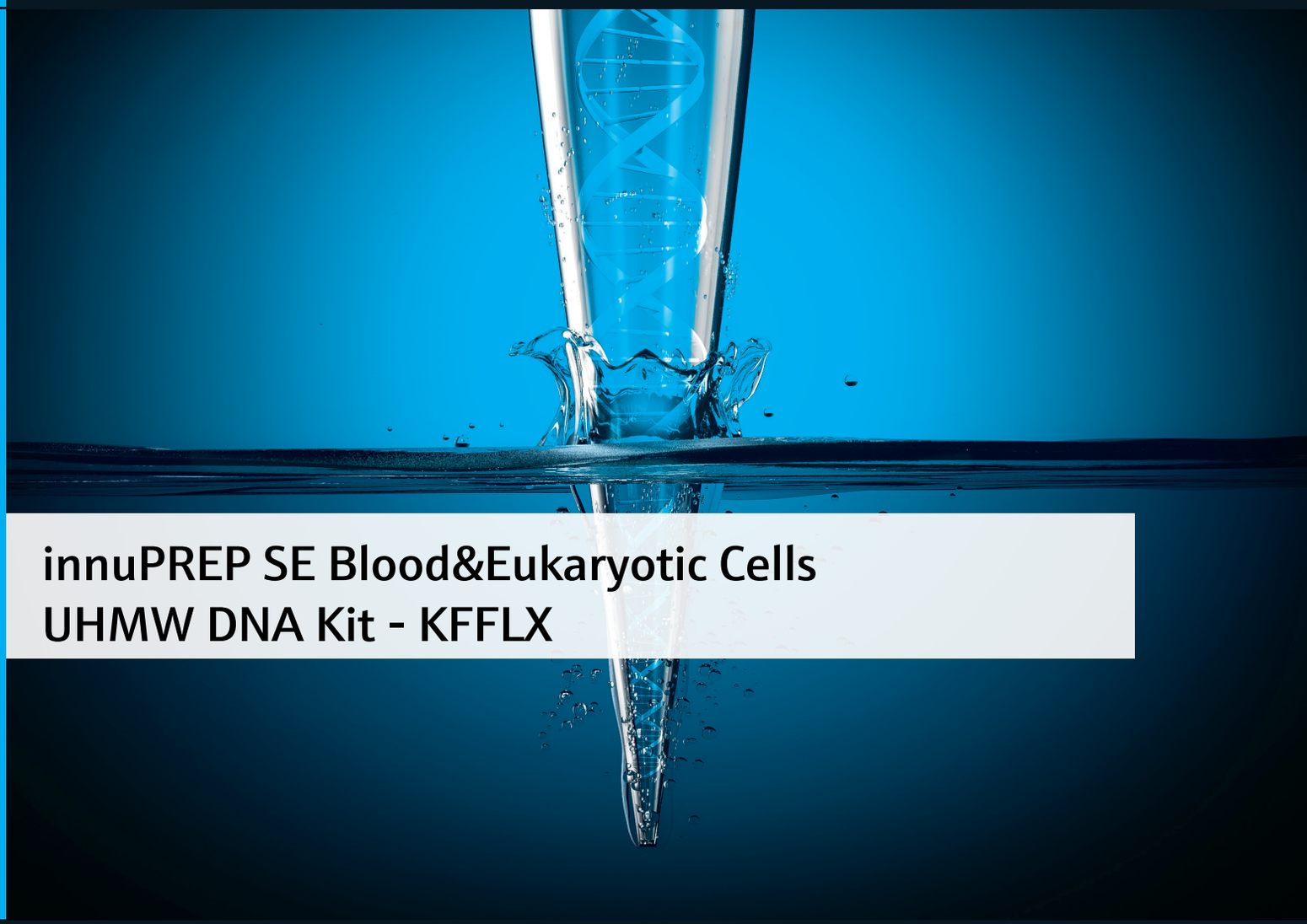
Instructions for Use
Life Science Kits & Assays



innuPREP SE Blood&Eukaryotic Cells UHMW DNA Kit - KFFLX

Innuscreen GmbH

Order No.:
845-KF-4096096 96 reactions
845-KF-4096480 480 reactions

Publication No.: HB_KF-4096_e_230207

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

Contents

1 Introduction

1.1 Intended use

The **innuPREP SE Blood&Eukaryotic Cells UHMW DNA Kit - KFFLX** has been designed for automated isolation of ultra-high molecular weight (UHMW) from cultivated eukaryotic cells or peripheral blood mononuclear cells (PBMC) derived from fresh or frozen blood stabilized with EDTA, citrate or heparin based on a patented technology.

For blood samples the procedure starts with the lysis of erythrocytes and the subsequent pelleting of the PBMC's. After addition of 1 x PBS, the cells are resuspended and transferred into the Deep Well Plate. For cultivated eukaryotic cells no preliminary steps are necessary. The extraction process is based on adsorption of the genomic DNA on so called Smart Modified Surfaces and it needs no magnetic particles for DNA binding. That means, the DNA binds directly 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 for subsequent downstream applications.

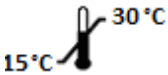
The whole extraction process just needs simple mixing up and down of the modified Tip Combs. The process is very fast and gives no limitation regarding the binding capacity. So, the kit is optimized to get a maximum of yield and quality.

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** 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 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 Blood&Eukaryotic Cells UHMW 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 Blood&Eukaryotic Cells UHMW 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. 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 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-4096096	845-KF-4096480
Ery Lysis Solution A (conc.)	100 ml	3 x 140 ml
Ery Lysis Solution B (conc.)	60 ml	3 x 100 ml
Lysis Solution CHV	25 ml	110 ml
Proteinase K	for 4 x 1.5 ml working solution	for 17 x 1.5 ml working solution
RNase A	500 µl	5 x 500 µl
Buffer H1	25 ml	110 ml
Buffer H2	6 ml	30 ml
Washing Solution MS (conc.)	54 ml	4 x 66 ml
Washing Solution ER	85 ml	450 ml
RNase free Water	25 ml	4 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)
- 70 % ethanol for washing plate 3
- ddH_2O; ultrapure for dissolving Proteinase K and for diluting Ery Lysis Solution A (conc.) and Ery Lysis Solution B (conc.)

7 Product specifications

1. Starting material:

- Eukaryotic cells (1 x 10^5–4 x 10^7)
- 0.5–5 ml whole blood (fresh or frozen) treated with EDTA, citrate or heparin

2. Time for automated extraction:

- 60 minutes

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.

- Use appropriate bottles and add the indicated volumes of **Ery Lysis Solution A (conc.)** to ddH_2O and mix thoroughly. Always keep the bottle firmly closed!

845-KF-4096096	Add 100 ml Ery Lysis Solution A (conc.) to 900 ml ddH_2O.
845-KF-4096480	Add 140 ml Ery Lysis Solution A (conc.) to 1260 ml ddH_2O.

- Use appropriate bottles and add the indicated volumes of **Ery Lysis Solution B (conc.)** to ddH_2O and mix thoroughly. Always keep the bottle firmly closed!

845-KF-4096096	Add 60 ml Ery Lysis Solution B (conc.) to 540 ml ddH_2O.
845-KF-4096480	Add 100 ml Ery Lysis Solution B (conc.) to 900 ml ddH_2O.

- Add the indicated volume of ethanol to each bottle of **Washing Solution MS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KF-4096096	Add 126 ml ethanol to 54 ml Washing Solution MS.
845-KF-4096480	Add 154 ml ethanol to 66 ml Washing Solution MS.

9 Protocols for isolation of gDNA from different starting materials

9.1 Protocol 1: Isolation from 0.5–5 ml whole blood

1. Dispense **Ery Lysis Solution A** according to the volume of whole blood sample (see table below) into a 15 ml tube.

Whole blood volume	Volume of Ery Lysis Solution A
0.5–1.0 ml	3.0 ml
2.0 ml	5.0 ml
3.0-5.0 ml	10.0 ml

2. Add 0.5–5.0 ml whole blood into the prepared 15 ml tube and mix by inverting 6 times.
3. Incubate 5–10 minutes at room temperature. Invert at least once during incubation time.

NOTE
For fresh blood (collected within 1–6 h before starting the extraction) increase incubation time to 20 minutes to ensure complete lysis of red blood cells.

4. Centrifuge for 5 minutes at 2,500 x g to pellet the PBMC.
5. Carefully discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet!

6. Add **5 ml Ery Lysis Solution B** to the PBMC pellet and vortex shortly or shake the tube vigorously to resuspend the cell pellet completely.
7. Centrifuge for 5 minutes at 2,500 x g to pellet the PBMC.
8. Carefully discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet! Use a paper towel to remove residual liquid as much as possible!

9. Add **130 µl PBS** to the cell pellet and resuspend the pellet as much as possible by pipetting up and down.

10. Transfer max. 220 µl of resuspended PBMC and transfer the cells into a well of the KF96 DW Plate (labeled with “Lysis Plate”).
11. Add 2**00 µl Lysis Solution CHV** and **30 µl Proteinase K** (for > 3.0 ml blood sample use 50 µl Proteinase K).

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

12. Proceed with “Automated extraction using KingFisher Flex” on p.12.

9.2 Protocol 2: Isolation from eucaryotic cells

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 5 minutes at 2,500 x g) and discard the supernatant as much as possible.
2. Add **130 µl PBS** to the cell pellet and resuspend the pellet as much as possible by pipetting up and down.
3. Transfer max. 220 µl of resuspended PBMC and transfer the cells into a well of the KF96 DW Plate (labeled with “Lysis Plate”).
4. Add **200 µl Lysis Solution CHV** and **40 µl Proteinase K**.

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

5. Proceed with “Automated extraction using KingFisher Flex” on p.12.

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	Cell pellet, Lysis Solution CHV and Proteinase K described as before
Deep Well	Washing Plate 1	800 µl Washing Solution MS
Deep Well	Washing Plate 2	800 µl Washing Solution MS
Deep Well	Washing Plate 3	800 µl 70% 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_4“ 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 **220 µl Buffer H1 and 40 µl Buffer H2** to each sample.

4. 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 the extraction protocol, the Elution Plate contains the isolated UHMW 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	
Insufficient lysis	Reduce amount of starting material.
Preparation without Binding Optimizer	Pay special attention that Binding Optimizer was added to the lysed sample!
High viscosity extracted DNA / Inhomogeneous DNA sample	
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