Instructions for Use
Life Science Kits & Assays

innuPREP cfDNA Mini Kit

Innuscreen GmbH

Order No.:
845-KS-3100100 100 reactions

Publication No.: HB_KS-3100_e_220927

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1 Introduction

1.1 Intended use

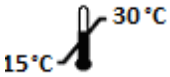
The **innuPREP cfDNA Mini Kit** has been designed as a very efficient tool for fast isolation of cell-free DNA (cfDNA) from different types of starting materials like serum, plasma or urine. The kit is intended for use by professional users. The extraction procedure is based on a new kind of chemistry, which combines an efficient lysis step with a subsequent efficient binding of cfDNA on a Spin Filter surface following washing of the bound cfDNA and finally eluting of the cfDNA. The recovery of DNA and the quality are excellent.

Further, the kit contains a Carrier RNA. Addition of Carrier RNA is recommended if extreme low amount of free-circulating DNA is expected. In this case the addition of Carrier RNA can increase the final yield. Using Real-time PCR as a downstream application has shown a benefit of 0.5 – 1 Ct-value. In all other cases the addition of Carrier RNA is not necessary.

The isolated DNA is suitable 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 with regard to other clinical or laboratory results.

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.
 N	**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).
- 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

The kit is shipped at ambient temperature.

Upon arrival, store lyophilized and dissolved **Proteinase K** at 4 °C to 8 °C.

Store lyophilized **Carrier RNA** at -22 to -18 °C! Aliquot dissolved **Carrier RNA** and store at -22 °C to -18 °C. Do not freeze and thaw **Carrier RNA** stock solution more than 3 times.

All other components of the innuPREP cfDNA Mini Kit 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 product has been produced 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 cfDNA Mini Kit 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 100
REF	845-KS-3100100
Lysis Solution VP	25 ml
Binding Solution V	2 x 25 ml
Proteinase K	for 2 x 1.5 ml working solution
Washing Solution A	2 x 35 ml
Washing Solution B2 (conc.)	2 x 16 ml
RNase-free Water	10 ml
Carrier RNA	for 2 x 1 ml working solution
Spin Filter	2 x 50
Receiver Tubes	8 x 50
Elution Tubes	2 x 50
Manual	1

6.2 Components not included in the kit

- 2.0 ml tubes
- ddH_2O for dissolving **Proteinase K**
- 96–99.8 % ethanol, non-denatured or methylated
- 1 x PBS

7 Product specifications

1. **Starting material:**
   - Serum, plasma or urine

2. **Time for isolation:**
   - Approximately 30 minutes

3. **Typical yield:**
   - Depends on type and amount of starting material
   - The extracted cfDNA can be used for a wide range of different molecular biology applications

8 Initial steps before starting

- Add 1.5 ml of ddH_2O to each vial of **Proteinase K**, mix thoroughly and store as described above.
- Add 1 ml of RNase-free Water to each vial of **Carrier RNA**, mix thoroughly by pipetting up and down and store as described above.
- Add 24 ml of 96 99.8 % ethanol to the bottle of Washing Solution B2 (conc.) and mix thoroughly. Always keep the bottle firmly closed!
- Heat thermal mixer or water bath at 70 °C.
- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.
- Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

9 Protocol: cfDNA extraction from up to 200 µl

1. Pipette **200 µl of Lysis Solution VP** into a 2.0 ml reaction tube. Add **200 µl of the sample** and **20 µl of Proteinase K**, mix vigorously by pulsed vortexing for 5 sec. Incubate at 70 ˚C for 15 minutes.

NOTE

Optional add 10 µl Carrier RNA to the sample **after adding** Lysis Solution VP. See "Intended use" if Carrier RNA is necessary to add or not!

NOTE

We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. No shaking will reduce the lysis efficiency.

NOTE

The sample volume is smaller than 200 µl then please fills it up to 200 µl with the 1× PBS.

After lysis centrifuge the reaction tube shortly to remove condensate from the lid of the tube.

2. Add **400 µl Binding Solution V** to the lysed sample, mix by brief vortexing for 10 seconds. Centrifuge the reaction tube shortly.

IMPORTANT

It is important that the sample and the **Binding Solution V** are mixed completely to get a homogeneous solution.

3. Apply the sample to the Spin Filter located in a Receiver Tube. Close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minutes. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

4. Open the Spin Filter and add **500 µl Washing Solution A**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
5. Open the Spin Filter and add **650 µl Washing Solution B2**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
6. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
7. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **50 µl RNase-free Water**. Incubate at room temperature for 2minute. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minute.

NOTE

The DNA can be eluted with a lower or a higher volume of RNase-free Water (depends on the expected yield of cell-free DNA). Elution with lower volumes of RNase-free Water increases the final concentration of cfDNA. Store the extracted cfDNA at 4 °C to 8 °C. For long time storage placing at −18 °C to -22 °C is recommended.

10 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. Reduce amount of starting material.
Low amount of extracted cfDNA	
Insufficient lysis	Increase lysis time! Reduce amount of starting material. Overloading reduces yield!
Insufficient mixing with Binding Solution V	Mix sample with Binding Solution V by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
Too much RNase-free Water was used in the elution step	Elute the cfDNA with lower volume of RNase-free Water

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