

Instructions for Use

Life Science Kits & Assays



innuPREP PME cfDNA Kit - PP Mini

Order No.:

845-PS-0080016	16 reactions
845-PS-0080096	96 reactions

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1 Introduction

1.1 Intended use

Free-circulating DNA in plasma or in urine is very interesting as diagnostic target. The amount of free-circulating DNA is usually very low and varies among different individuals. Further, the free-circulating DNA is present as short fragments, usually smaller than 1000 nt. Because of these facts, the extraction of cell-free-circulating DNA is difficult. Commercially available kits use standard nucleic acid extraction procedures based on sample lysis, binding the nucleic acids on a solid material, washing and elution of nucleic acids. Because of the high sample volume, the procedures are very time and work consuming and need a lot of reagents. The innuPREP PME cfDNA Kit – PP Mini is based on new technology, called: PME – Polymer Mediated Enrichment. The procedure does not start with sample lysis, like commonly used methods or kits. The first step is capturing of free-circulating DNA with a special polymer. Subsequently the captured free-circulating DNA is dissolved in a special lysis buffer and then the DNA is extracted. There are 2 different capturing variants possible with this kit.

1. PME – cfDNA capture followed by centrifugation
2. PME – cfDNA capture and simultaneous binding to magnetic beads - no centrifugation

The kit combines a unique patented technology for enrichment of biomolecules with a very efficient extraction of DNA using magnetic particles. The extraction procedure takes place on the magnetic particle processor PurePrep Mini and allows the parallel and flexible extraction of 1 up to 16 samples.

The extraction process starts with 10 ml of urine or 5 ml plasma sample.



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.

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.4).
- Work 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 that 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, immediately flush eyes or skin with a large amount of water.



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.

Store lyophilized and dissolved **Proteinase K**, **MAG Suspension F**, **Enrichment Reagent VCR-1** and dissolved **Carrier RNA** at 4 °C to 8 °C.

Store lyophilized **Carrier RNA** at -22 °C to -18 °C.

All other components of the **innuPREP PME cfDNA Kit – PP Mini** 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.

For further information see chapter "Kit components" (→ p. 8).

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 kit 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 PME cfDNA Kit – PP Mini** 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 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 16	▽ Σ 96
REF	845-PS-0080016	845-PS-0080096
MAG Suspension F	0.25 ml	1.1 ml
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution
Carrier RNA	for 1 x 1.0 ml working solution	for 1 x 1.0 ml working solution
Lysis Solution RL	8 ml	50 ml
Binding Solution V	8 ml	60 ml
Washing Solution HS (conc.)	15 ml	90 ml
Washing Solution LS (conc.)	6 ml	35 ml
Enrichment Reagent VCR-1	2 x 1.2 ml	7 x 1.2 ml
Enrichment Reagent VCR-2	2 x 2 ml	20 ml
RNase-free Water	2 ml	12 ml
Manual	1	1

6.2 Components not included in the kit

- 15 ml tubes
- 2.0 ml or 1.5 ml reaction tubes
- ddH₂O for dissolving **Proteinase K**
- RNase-free Water for dissolving **Carrier RNA**
- 96–99.8 % ethanol, non-denatured or methylated

7 Product specifications

1. **Starting material:**
 - 10 ml urine
 - 5 ml plasma
2. **Time for isolation:**
 - Approximately 90 minutes including all steps

8 Initial steps before starting

- Add the indicated amount of ddH₂O to **Proteinase K**, mix thoroughly and store as described above.

845-PS-0080016/96	Add 1.5 ml ddH ₂ O to lyophilized Proteinase K.
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- Add the indicated amount of RNase-free water to **Carrier RNA**, mix thoroughly and store as described above.

845-PS-0080016/96	Add 1.0 ml RNase-free Water to Carrier RNA.
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- Add the indicated amount of absolute ethanol to **Washing Solution HS (conc.)**, mix thoroughly and store as described above.

845-PS-0080016	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
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845-PS-0080096	Add 90 ml ethanol to 90 ml Washing Solution HS (conc.).
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- Add the indicated amount of absolute ethanol to **Washing Solution LS (conc.)**, mix thoroughly and store as described above.

845-PS-0080016	Add 24 ml ethanol to 6 ml Washing Solution LS (conc.).
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845-PS-0080096	Add 140 ml ethanol to 35 ml Washing Solution LS (conc.).
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- Centrifugation steps should be carried out at room temperature.

9 Protocol 1: Isolation of cfDNA from 5 ml plasma sample using centrifugation protocol

9.1 Preliminary steps

Transfer 5 ml plasma into a 15 ml tube. Centrifuge at 5.000 x g for 10 minutes and transfer the supernatant into a new 15 ml tube.

9.2 Enrichment of cfDNA using a centrifuge

1. Add 75 µl VCR-1 and 150 µl VCR-2 and mix the tube vigorously by shaking it.
2. Incubate the tube at room temperature for 10 minutes.
3. Centrifuge the tube at 5.000 x g for 10 minutes. Remove the supernatant carefully.
4. Add 5 ml ddH₂O and invert the tube several times. Do not resuspend the pellet. Centrifuge the tube at 5.000 x g for 5 minutes. Remove as much of the supernatant as possible (using a pipet tip)
5. Add 450 µl Lysis Solution RL, 30 µl Proteinase K and 10 µl Carrier RNA. Incubate the tube at room temperature for 5 minutes.

NOTE

A premixture of Lysis Solution RL / PK and Carrier RNA will be possible!

6. Resuspend the pellet completely using a pipet.

10 Protocol 2: Isolation of cfDNA from 10 ml urine sample using centrifuge protocol

10.1 Preliminary steps

Transfer 10 ml urine sample into a 15 ml tube. Centrifuge at 5.000 x g for 10 minutes and transfer the supernatant into a new 15 ml tube.

10.2 Enrichment of cfDNA using a centrifuge

1. Add 75 µl VCR-1 and 150 µl VCR-2 and mix the tube vigorously by shaking it.
2. Incubate the tube at room temperature for 10 minutes.
3. Centrifuge the tube at 5.000 x g for 10 minutes. Remove the supernatant carefully.
4. Add 5 ml ddH₂O and invert the tube several times. Do not resuspend the pellet. Centrifuge the tube at 5.000 x g for 5 minutes. Remove as much of the supernatant as possible (using a pipet tip)
5. Add 450 µl Lysis Solution RL and 30 µl PK and 10 µl Carrier RNA. Incubate the tube at room temperature for 5 minutes.

NOTE

A premixture of Lysis Solution RL / PK and Carrier RNA will be possible!

6. Resuspend the pellet completely using a pipet.

11 Protocol 3: Isolation of cfDNA from 5 ml plasma sample using bead protocol

11.1 Preliminary steps

Transfer 5 ml plasma into a 15 ml tube. Centrifuge at 5.000 x g for 10 minutes and transfer the supernatant into a new 15 ml tube.

11.2 Enrichment of cfDNA using beads

1. Add 75 µl VCR-1 and 5 µl Mag Suspension F to the sample. Shake the tube vigorously.

NOTE

Vortex the MAG Suspension F for 30 seconds before use!

2. Add 150 µl VCR-2 and mix the tube vigorously by shaking it.
3. Incubate the tube at room temperature for 10 minutes. Invert the tube several times during incubation.
4. Place the tube into a magnetic rack (15 ml) or any other magnetic particle separation equipment for 15 ml tubes. Separate the beads from the supernatant and remove the supernatant carefully as complete as possible.
5. Leave the tube in the rack. Add 5 ml ddH₂O and invert the tube staying in the rack several times. Do not resuspend the pellet. Remove as much as possible of the supernatant (using a pipet tip)
6. Add 450 µl Lysis Solution RL and 30 µl PK and 10 µl Carrier RNA. Incubate the tube at room temperature for 5 minutes.

NOTE

A premixture of Lysis Solution RL / PK and Carrier RNA will be possible!

7. Resuspend the bead-pellet completely using a pipet.

12 Protocol 4: Isolation of cfDNA from 10 ml urine sample using bead protocol

12.1 Preliminary steps

Transfer 5 ml urine into a 15 ml tube. Centrifuge at 5.000 x g for 10 minutes and transfer the supernatant into a new 15 ml tube.

12.2 Enrichment of cfDNA using beads

1. Add 75 µl VCR-1 and 5 µl Mag Suspension F to the sample. Shake the tube vigorously.

NOTE

Vortex the MAG Suspension F for 30 seconds before use!

2. Add 150 µl VCR-2 and mix the tube vigorously by shaking it.
3. Incubate the tube at room temperature for 10 minutes. Invert the tube several times during incubation.
4. Place the tube in a magnetic rack (15 ml) or another magnetic particle separation equipment for 15 ml tubes. Separate the beads from the supernatant and remove the supernatant carefully as complete as possible.
5. Leave the tube in the rack. Add 5 ml ddH₂O and invert the tube staying in the rack several times. Let the tube stay in the rack. Do not resuspend the pellet. Remove as much of the supernatant as possible (using a pipet tip).
6. Add 450 µl Lysis Solution RL and 30 µl PK and 10 µl Carrier RNA. Incubate the tube at room temperature for 5 minutes.

NOTE

A premixture of Lysis Solution RL / PK and Carrier RNA will be possible!

7. Resuspend the bead-pellet completely using a pipet.

13 Automated extraction using PurePrep Mini

13.1 Prefilling of the DW Plate or the DW Strips

Cavity of DW Plate/Strip	Content
Cavity 1	A: 400 µl resuspended sample B: 400 µl resuspended bead-pellet
Cavity 2	800 µl Washing Solution HS
Cavity 3	800 µl Washing Solution HS
Cavity 4	800 µl Washing Solution LS
Cavity 5	800 µl Washing Solution LS
Cavity 6	100 µl RNase free Water

13.2 Loading filled Deep Well Plate/Strips to the PurePrep Mini and plug in the Tip Combs

NOTE

- When using strip (strips), the strip is inserted into the tray. In total, a maximum of 8 strips can be used in one extraction-run.
- The tip combs always dip staggered into the Strips.

Left tray side: Tip 1, 3, 5, 7

Right tray side: Tip 2, 4, 6, 8.

- It is recommended to mark the tips used for the extraction so that they are not used more than once
-

1. Select the protocol
"CFDNA" and start the run.
2. The automated extraction process starts with sample lysis. After sample lysis the automated run stops.
3. After the device has stopped, take the Plate/Strip out of the device and add:

A: 5 µl of well mixed MAG Suspension F and 450 µl of Binding Solution V to the lysed samples without beads

B: 450 µl of Binding Solution V to the lysed samples with beads.

NOTE

Vortex the MAG Suspension F for 30 seconds before use!

IMPORTANT NOTE

After finishing the extraction protocol, the Elution Plate contains the isolated DNA. Store the DNA under adequate conditions.

We recommend storing the extracted DNA for longer use at -22°C to -18°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 into a new plate.

14 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Insufficient lysis	Reduce amount of starting material. Overloading reduces yield!
Low concentration of extracted DNA	
Too much RNase-free Water was used for the elution step	Elute the DNA in a lower volume of RNase-free Water (min. 80 µl).

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