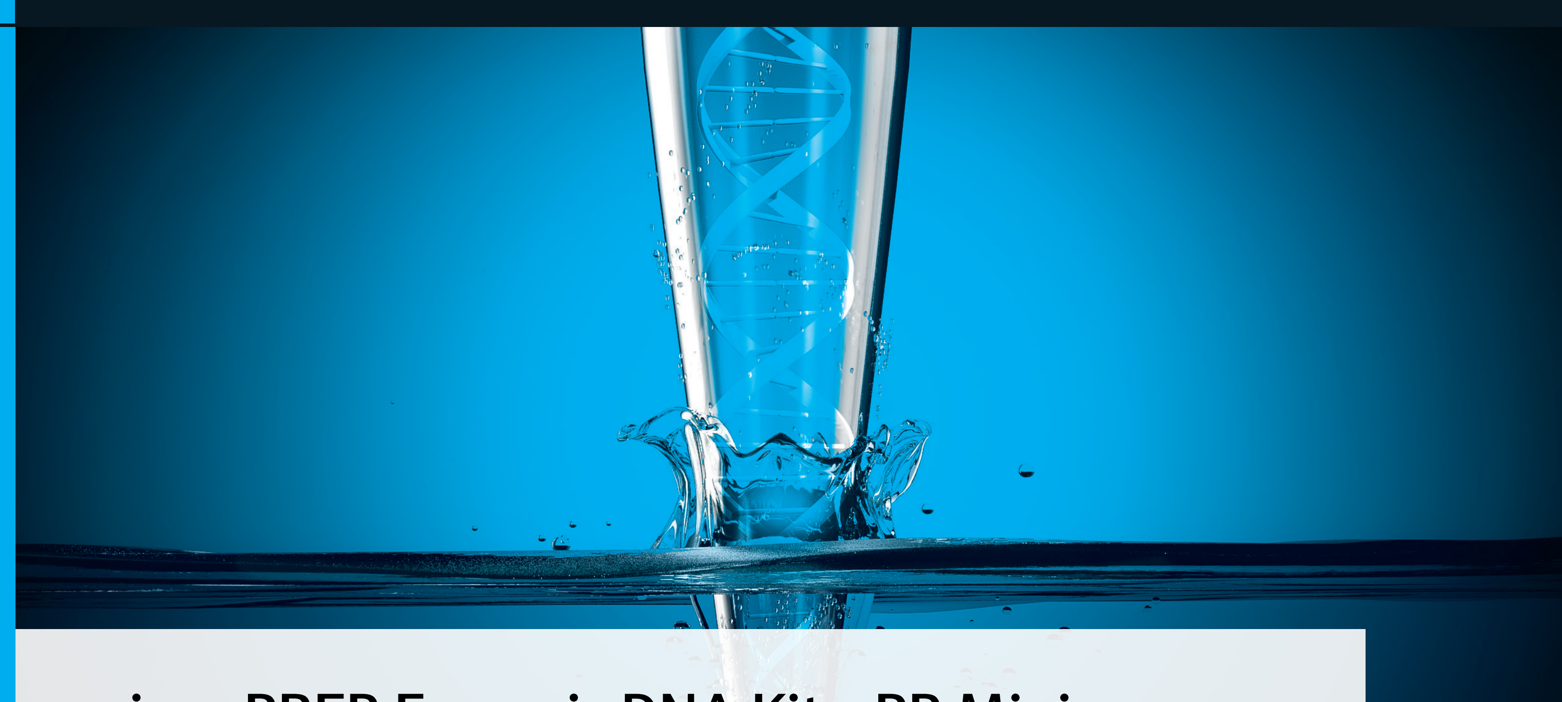
Instructions for Use

Life Science Kits & Assays



innuPREP Forensic DNA Kit - PP Mini

Order No.:

845-PS-0050016	16 reactions
845-PS-0050096	96 reactions

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

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1 Introduction

1.1 Intended use

The **innuPREP Forensic DNA Kit - PP Mini** has been designed for the automated isolation of DNA from small amounts of different types of forensic samples like hair or hair roots, stains of blood, saliva or sperm, fingernails, cigarette butts, bubble gum, buccal swabs, stamps and envelopes as well as fingerprints on different surfaces on the PurePrep Mini. The extraction procedure is based on a new kind of chemistry.

The extraction procedure takes place on the magnetic particle processor PurePrep Mini and allows the parallel and flexible extraction of 1 to 16 samples.

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 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, 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** and **MAG Suspension F** at 4 °C to 8 °C.

All other components of the 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.

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 Forensic DNA 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.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
This kit is for research use only!

6 Kit components

6.1 Components included in the kit

	Σ 16	Σ 96
REF	845-PS-0050016	845-PS-0050096
Lysis Solution CBV	10 ml	60 ml
MAG Suspension F	0.25 ml	1.1 ml
Binding Solution SBS	8 ml	45 ml
Proteinase K	2 x for 0.3 ml working solution	2 x for 1.5 ml working solution
Washing Solution A	30 ml	180 ml
Washing Solution B2 (conc.)	10 ml	48 ml
Washing Solution ER	17 ml	85 ml
Elution Buffer	6 ml	25 ml
Manual	1	1

6.2 Components not included in the kit

- 1.5 ml tubes
- 96 %–99.8 % ethanol (molecular biology grade, undenatured)
- ddH_2O; ultrapure for dissolving Proteinase K
- 1 M DTT solution
- 96-well-plates or strips and tip combs for PurePrep Mini

7 Product specifications

1. Starting material:

- Swabs from different surfaces (e.g. cups, bottles, fingerprints)
- Blood samples
- Sperm samples
- Hair, hair roots or barb hairs
- Envelopes
- Fingernails
- Cigarette butts or paper
- Chewing gum

2. Time for automated extraction protocol on PurePrep Mini:

Approx. 20 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-PS-0050016	Add 0.3 ml ddH_2O to lyophilized **Proteinase K**.
845-PS-0050096	Add 1.5 ml ddH_2O to lyophilized **Proteinase K**.

- Add the indicated volume of absolute ethanol to **Washing Solution B2 (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-PS-0050016	Add 15 ml ethanol to 10 ml **Washing Solution B2**.
845-PS-0050096	Add 72 ml ethanol to 48 ml **Washing Solution B2**.

9 Sample Preparation

9.1 Protocol 1: Extraction from swab samples from different surfaces (cups, bottles, fingerprints etc.)

NOTE

To get maximum yield of DNA it is essential to leave the swab in the 1.5 ml tube during the complete lysis time. It is possible to cut off the shaft of the swab to be able to close the lid of the tube. The removal of the swab from the tube ahead of time will lead to a dramatically reduced final yield!

1. Place the swab into a 1.5 ml tube and add **500 µl Lysis Solution CBV** and **20 µl Proteinase K.**
2. Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 55 °C for 20 minutes.

NOTE

Assure that the swab is in the Lysis Solution during the lysis time! We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the lysis efficiency!

3. After lysis time remove the swab from the 1.5 ml tube and squeeze the swab on the wall of the tube to remove all **Lysis Solution CBV** from the swab.
4. Transfer **400 µl** of the sample into the first well of a Deep Well Plate/Strip and add **400 µl Binding Solution SBS** and **10 µl Mag Suspension F**.
5. Proceed with “Automated extraction using PurePrep Mini” on p.16.

9.2 Protocol 2: Extraction from sperm samples, hair roots, barb hairs, finger nails

1. Cut the material into small pieces and transfer it into a 1.5 ml reaction tube and add **400 µl Lysis Solution CBV, 20 µl Proteinase K** and **30 µl DTT** solution (1M; not provided).

2. Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 55 °C for at least 3 hours.

NOTE
Assure that the swab is in the Lysis Solution during the lysis time! We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the lysis efficiency!

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3. Centrifuge the 1.5 ml tube at 10,000 x g for 1 minute to spin down unlysed material.

4. Transfer **400 µl** of the sample into the first well of a Deep Well Plate/Strip and add **400 µl Binding Solution SBS** and **10 µl Mag Suspension F**.

5. Proceed with “Automated extraction using PurePrep Mini” on p.16.

9.3 Protocol 3: Extraction from blood spots on surfaces (like clothes), envelopes, cigarette butts or paper and chewing gum

1. Cut the material into small pieces and transfer it into a 1.5 ml reaction tube and add **400 µl Lysis Solution CBV, 30 µl Proteinase K** and **30 µl DTT** solution (1M; not provided).

2. Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 55 °C for at least 2 hours.

NOTE
Assure that the swab is in the Lysis Solution during the lysis time! We recommend using a shaking platform (thermal mixer, water bath or

another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the lysis efficiency!

3. Centrifuge the 1.5 ml tube at 10,000 x g for 1 minute to spin down unlysed material.
4. Transfer **400 µl** of the sample into the first well of a Deep Well Plate/Strip and add **400 µl Binding Solution SBS** and **10 µl Mag Suspension F**.
5. Proceed with "Automated extraction using PurePrep Mini" on p.16.

9.4 Protocol 4: Extraction from saliva samples

1. Transfer 200 µl saliva into a 1.5 ml tube and add 2**00 µl Lysis Solution CBV** and **20 µl Proteinase K.**
2. Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 55 °C for at least 30 minutes.

NOTE
Assure that the swab is in the Lysis Solution during the lysis time! We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the lysis efficiency!

3. Transfer **400 µl** of the sample into the first well of a Deep Well Plate/Strip and add **400 µl Binding Solution SBS** and **10 µl Mag Suspension F**.
4. Proceed with "Automated extraction using PurePrep Mini" on p.16.

9.5 Protocol 5: Extraction from whole blood or buffy coat samples

1. Transfer 100 µl blood or buffy coat into a 1.5 ml tube and add **300 µl Lysis Solution CBV** and **30 µl Proteinase K.**

2. Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 55 °C for at least 30 minutes.

NOTE

Assure that the swab is in the Lysis Solution during the lysis time! We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the lysis efficiency!

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3. Transfer **400 µl** of the sample into the first well of a Deep Well Plate/Strip and add **400 µl Binding Solution SBS** and **10 µl Mag Suspension F**.

4. Proceed with “Automated extraction using PurePrep Mini” on p.16.

9.6 Protocol 6: Extraction from dry buccal swabs

NOTE
To get maximum yield of DNA it is essential to leave the swab in the 1.5 ml tube during the complete lysis time. It is possible to cut the shaft off the swab, to be able to close the lid of the tube. The removal of the swab from the tube ahead of time will lead to a dramatically reduced final yield!

1. Place the swab into a 1.5 ml tube and add 5**00 µl Lysis Solution CBV** and **20 µl Proteinase K.**
2. Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 55 °C for 20 minutes.

NOTE
Assure that the swab is in the Lysis Solution during the lysis time! We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the lysis efficiency!

3. After lysis time remove the swab from the 1.5 ml tube and squeeze the swab on the wall of the tube to remove all **Lysis Solution CBV** from the swab.
4. Transfer **400 µl** of the sample into the first well of a Deep Well Plate/Strip and add **400 µl Binding Solution SBS** and **10 µl Mag Suspension F**.
5. Proceed with "Automated extraction using PurePrep Mini" on p.16.

9.7 Protocol 7: Extraction from buccal swabs in storage buffer

1. Squeeze out the swab and remove the swab. Transfer 200 µl of the storage buffer into a 1.5 ml tube and add **200 µl Lysis Solution CBV** and **20 µl Proteinase K.**

2. Mix vigorously by pulsed vortexing for 5 seconds. Incubate at 55 °C for 30 minutes.

NOTE
Assure that the swab is in the Lysis Solution during the lysis time! We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during the incubation. No shaking will reduce the lysis efficiency!

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3. Transfer **400 µl** of the sample into the first well of a Deep Well Plate/Strip and add **400 µl Binding Solution SBS** and **10 µl Mag Suspension F**.

4. Proceed with "Automated extraction using PurePrep Mini" on p.16.

10 Automated extraction using PurePrep Mini

10.1 Prefilling of the DW Plate or the DW Strips

Cavity of DW Plate/Strip	Content
Cavity 1	Lysed Sample + 400 µl Binding Solution SBS + 10 µl Mag Suspension F
Cavity 2	800 µl Washing Solution A
Cavity 3	800 µl Washing Solution A
Cavity 4	800 µl Washing Solution B2
Cavity 5	800 µl Washing Solution ER
Cavity 6	100 µl Elution Buffer

NOTE

Mix the **MAG Solution F** well by vortexing for 1 minute.

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

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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

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1. Select the protocol

   “GDNA1“ and start the run.
2. After finishing the extraction protocol, the Cavity 6 contains the isolated DNA.

IMPORTANT NOTE

After finishing the extraction protocol, the last cavity of the Plate/Strip contains the isolated DNA. Store the DNA under adequate conditions.

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.

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11 Troubleshooting

Problem / probable cause	Comments and suggestions
Poor lysis of starting material	
Insufficient disruption or homo-genization	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 homo-genization	Reduce amount of starting material. Over-loading 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.

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