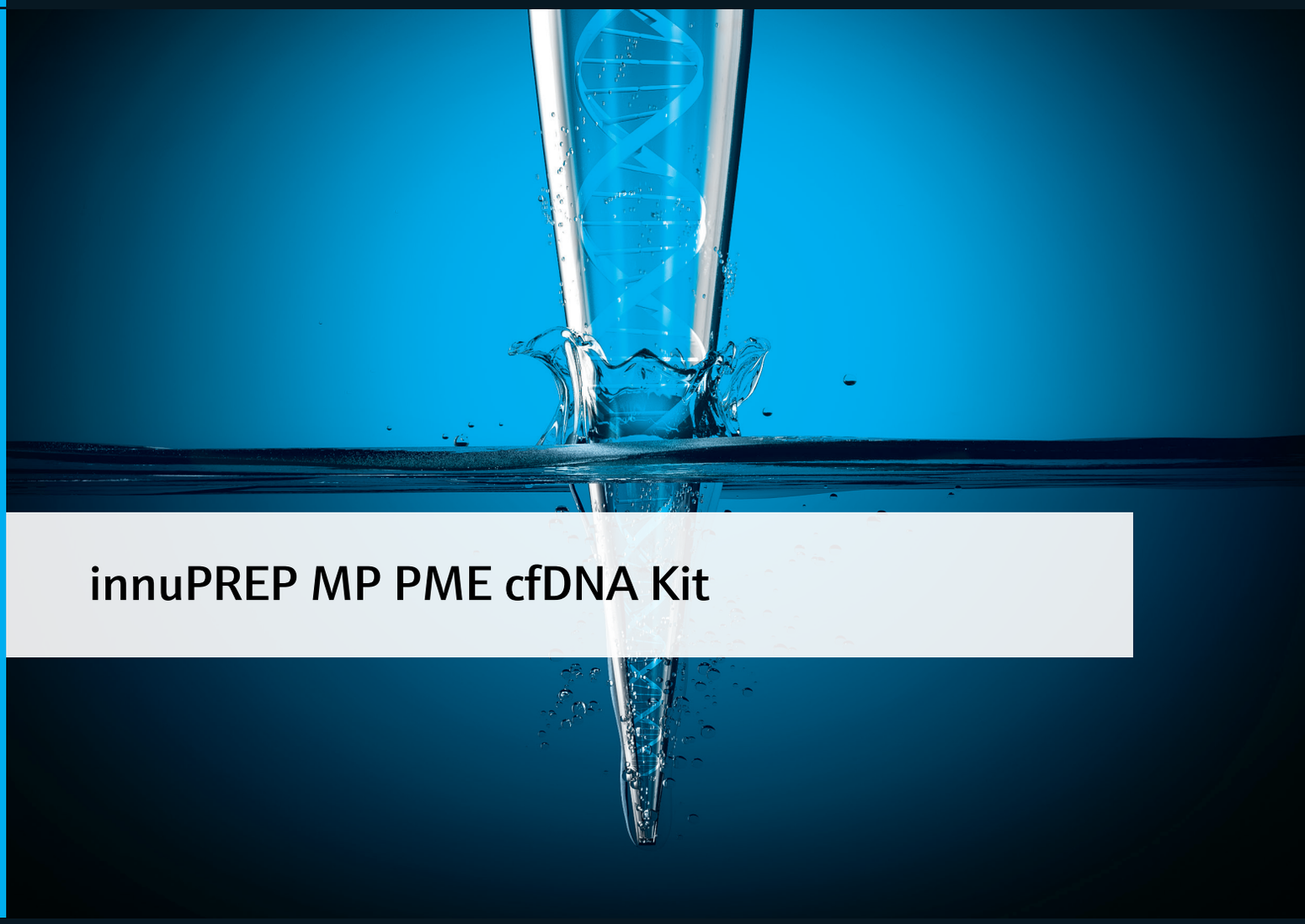
Instructions for Use
Life Science Kits & Assays



innuPREP MP PME cfDNA Kit

Innuscreen GmbH

Order No.:

845-KS-4930010	10 reactions
845-KS-4930050	50 reactions

Publication No.: HB_KS-4930_e_231113

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

Contents

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 MP PME cfDNA Kit** is based on a 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.

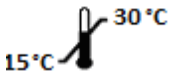
1. PME – cfDNA capture followed by centrifugation
2. PME – cfDNA capture and simultaneous binding to magnetic beads - no centrifugation

The kit combines a unique patented technology for enrichment of biomolecules with a very efficient extraction of DNA using magnetic particles.
The extraction 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 can be used with potentially 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 and 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 MP PME cfDNA 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 are at 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 MP PME cfDNA 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. 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 10	Σ 50
REF	845-KS-4930010	845-KS-4930050
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	6 ml	25 ml
Binding Solution V	5 ml	25 ml
Washing Solution HS (conc.)	6 ml	30 ml
Washing Solution LS (conc.)	4 ml	20 ml
Enrichment Reagent VCR-1	1 x 1.2 ml	4 x 1.2 ml
Enrichment Reagent VCR-2	2 ml	10 ml
RNase-free Water	2 ml	6 ml
Manual	1	1

6.2 Components not included in the kit

- 50 ml tubes
- 2.0 ml or 1.5 ml reaction tubes
- ddH_2O for dissolving **Proteinase K**
- RNase-free Water for dissolving **Carrier RNA**
- 96–99.8 % ethanol, non-denatured or methylated
- Magnetic Rack (6fold) for 1.5 - 2.0 ml Tubes (IST Innuscreen; 845-MR-0600001)
- Magnetic Rack for 15 ml Tubes (IST Innuscreen; 845-MG-2000015)

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 ddH_2O to **Proteinase K**, mix thoroughly and store as described above.

845-KS-4930010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-4930050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of RNase-free Water to **Carrier RNA**, mix thoroughly and store as described above.

845-KS-4930010/50	Add 1.0 ml RNase-free Water to Carrier RNA.

- Add the indicated amount of absolute ethanol to **Washing Solution HS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed!

845-KS-4930010	Add 6 ml ethanol to 6 ml Washing Solution HS (conc.).
845-KS-4930050	Add 30 ml ethanol to 30 ml Washing Solution HS (conc.).

- Add the indicated amount of absolute ethanol to **Washing Solution LS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed!

845-KS-4930010	Add 16 ml ethanol to 4 ml Washing Solution LS (conc.).
845-KS-4930050	Add 80 ml ethanol to 20 ml Washing Solution LS (conc.).

- Heat thermal mixer or water bath to 60 °C (later to 50 °C).
- Centrifugation steps should be carried out at room temperature.

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

9.4 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.

9.5 Enrichment of cfDNA using a centrifuge

5. Add **75 µl VCR-1** and **150 µl VCR-2** and mix the tube vigorously by shaking it.
6. Incubate the tube at room temperature for 10 minutes.
7. Centrifuge the tube at 5.000 x g for 10 minutes. Remove the supernatant carefully.
8. Add **5 ml ddH_2O** 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)
9. Add **450 µl Lysis Solution RL**, **30 µl Proteinase K and 10 µl Carrier RNA**. Incubate the tube at room temperature for 5 minutes.

9.6 Extraction of cfDNA

1. Resuspend the pellet completely using a pipet.
2. Transfer the resuspended sample into a 1.5 ml reaction tube. Vortex shortly. Place the reaction tube into a thermal mixer and incubate under continuous shaking (500 rpm) for 20 minutes at 60°C.
3. Add **450 µl Binding Solution V** and **5 µl MAG Suspension F** to the lysed sample.

NOTE
Vortex the MAG Suspension F for 30 seconds before use! The Binding Solution V is viscous, please pipette carefully.

4. Mix the sample completely by vortexing for 5 seconds. Incubate the reaction tube at room temperature for 5 minutes for the binding of the nucleic acids to the magnetic particles.

5. Place the reaction tube into a magnetic rack (1.5-2.0 ml). Separate the beads from the supernatant and remove the supernatant as completely as possible using a pipet tip.

6. Add 500 µl **Washing Solution HS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of the beads and remove the **Washing Solution HS**.

7. Add 500 µl **Washing Solution HS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of beads and remove the **Washing Solution HS**.

8. Add 750 µl **Washing Solution LS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the **Washing Solution LS**.

9. Add 750 µl **Washing Solution LS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the **Washing Solution LS**.

NOTE
After the last washing step remove **Washing Solution LS** as completely as possible!

10. Place the open reaction tube with the magnetic beads into a thermal mixer at 50 °C for 5 minutes.

NOTE
The drying step is important for all following downstream applications. The ethanol must be removed completely!

11. Add **40 µl – 100 µl RNase-free water**, resuspend the magnetic particles completely and incubate at 50°C in a thermal mixer under continuous shaking (500 rpm) for 5 minutes.

12. Afterwards perform magnetic separation of beads. Transfer the eluted DNA into a new 1.5 ml reaction tube.

NOTE
The elution volume depends on expected amount of target nucleic acid.

10 Protocol 2: Isolation of cfDNA from 10 ml urine sample using centrifugation 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 ddH_2O** 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.

10.3 Extraction of cfDNA

1. Resuspend the pellet completely using a pipet.
2. Transfer the resuspended sample into a 1.5 ml reaction tube. Vortex shortly. Place the reaction tube into a thermal mixer and incubate under continuous shaking (500 rpm) for 20 minutes at 60°C.
3. Add **450 µl Binding Solution V** and **5 µl MAG Suspension F** to the lysed sample.

NOTE
Vortex the MAG Suspension F for 30 seconds before use! The Binding Solution V is viscous, please pipette carefully.

4. Mix the sample completely by vortexing for 5 seconds. Incubate the reaction tube at room temperature for 5 minutes for the binding of the nucleic acids to the magnetic particles.

5. Place the reaction tube into a magnetic rack (1.5-2.0 ml). Separate the beads from the supernatant and remove the supernatant as completely as possible using a pipet tip.

6. Add 500 µl **Washing Solution HS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of the beads and remove the **Washing Solution HS**.

7. Add 500 µl **Washing Solution HS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of beads and remove the **Washing Solution HS**.

8. Add 750 µl **Washing Solution LS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the **Washing Solution LS**.

9. Add 750 µl **Washing Solution LS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the **Washing Solution LS**.

NOTE
After the last washing step remove **Washing Solution LS** as completely as possible!

10. Place the open reaction tube with the magnetic beads into a thermal mixer at 50 °C for 5 minutes.

NOTE
The drying step is important for all following downstream applications. The ethanol must be removed completely!

11. Add **40 µl – 100 µl RNase-free Water**, resuspend the magnetic particles completely and incubate at 50°C in a thermal mixer under continuous shaking (500 rpm) for 5 minutes.

NOTE
The elution volume depends on expected amount of target nucleic acid.

12. Perform magnetic separation of beads. Transfer the eluted DNA into a new 1.5 ml reaction tube.

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 completely as possible.
5. Leave the tube in the rack. Add **5 ml ddH_2O** and invert the tube several times staying 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**, **30 µl Proteinase K and 10 µl Carrier RNA**. Incubate the tube at room temperature for 5 minutes.

11.3 Extraction of cfDNA

1. Resuspend the bead-pellet completely using a pipet.
2. Transfer the resuspended beads into a 1.5 ml reaction tube. Vortex shortly. Place the reaction tube into a thermal mixer and incubate under continuous shaking (500 rpm) for 20 minutes at 60°C.

3. Add **450 µl Binding Solution V**. Mix the sample completely by vortexing for 5 seconds. Incubate the reaction tube at room temperature for 5 minutes for the binding of the nucleic acids to the magnetic particles.

4. Place the reaction tube in a magnetic rack (available from IST Innuscreen). Separate the beads from the supernatant and remove the supernatant as completely as possible using a pipet tip.

5. Add 500 µl **Washing Solution HS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of the beads and remove the **Washing Solution HS**.

6. Add 500 µl **Washing Solution HS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of beads and remove the **Washing Solution HS**.

7. Add 750 µl **Washing Solution LS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the **Washing Solution LS**.

8. Add 750 µl **Washing Solution LS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the **Washing Solution LS**.

NOTE
After the last washing step remove **Washing Solution LS** as completely as possible!

9. Place the open reaction tube with the magnetic beads into a thermal mixer at 50 °C for 5 minutes.

NOTE
The drying step is important for all following downstream applications. The ethanol must be removed completely!

10. Add **40 µl – 100 µl RNase-free Water**, resuspend the magnetic particles completely and incubate at 50°C in a thermal mixer under continuous shaking (500 rpm) for 5 minutes.

NOTE
The elution volume depends on expected amount of target nucleic acid.

11. Perform magnetic separation of beads. Transfer the eluted DNA into a new 1.5 ml reaction tube.

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

12.1 Preliminary steps

Transfer 10 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 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 completely as possible.
5. Leave the tube in the rack. Add **5 ml ddH_2O** and invert the tube several times staying 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**, **30 µl Proteinase K and 10 µl Carrier RNA**. Incubate the tube at room temperature for 5 minutes.

12.3 Extraction of cfDNA

1. Resuspend the bead-pellet completely using a pipet.
2. Transfer the resuspended beads into a 1.5 ml reaction tube. Vortex shortly. Place the reaction tube into a thermal mixer and incubate under continuous shaking (500 rpm) for 20 minutes at 60°C.

3. Add **450 µl Binding Solution V.** Mix the sample completely by vortexing for 5 seconds. Incubate the reaction tube at room temperature for 5 minutes for the binding of the nucleic acids to the magnetic particles.

4. Place the reaction tube in a magnetic rack (available from IST Innuscreen). Separate the beads from the supernatant and remove the supernatant as complete as possible using a pipet tip.

5. Add 500 µl **Washing Solution HS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of the beads and remove the **Washing Solution HS**.

6. Add 500 µl **Washing Solution HS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform the magnetic separation of beads and remove the **Washing Solution HS**.

7. Add 750 µl **Washing Solution LS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the **Washing Solution LS**.

8. Add 750 µl **Washing Solution LS** and wash the magnetic particles by vortexing or by pipetting up and down. Perform magnetic separation of beads and remove the **Washing Solution LS**.

NOTE
After the last washing step remove **Washing Solution LS** as completely as possible!

9. Place the open reaction tube with the magnetic beads into a thermal mixer at 50 °C for 5 minutes.

NOTE
The drying step is important for all following downstream applications. The ethanol must be removed completely!

10. Add **40 µl – 100 µl RNase-free Water**, resuspend the magnetic particles completely and incubate at 50°C in a thermal mixer under continuous shaking (500 rpm) for 5 minutes.

NOTE
The elution volume depends on expected amount of target nucleic acid.

11. Perform magnetic separation of beads. Transfer the eluted DNA into a new 1.5 ml reaction tube.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Insufficient lysis	Increase lysis time! Reduce amount of starting material. Overloading reduces yield!
Incomplete elution	Prolong the incubation time with RNase-free Water to 5 minutes or repeat elution. Take a higher volume of RNase-free Water.
Insufficient mixing with Binding Solution V	Mix sample with Binding Solution V by pipetting.
Low concentration of extracted DNA	
Too much RNase-free Water was used for the elution step	Elute the DNA with lower volume

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