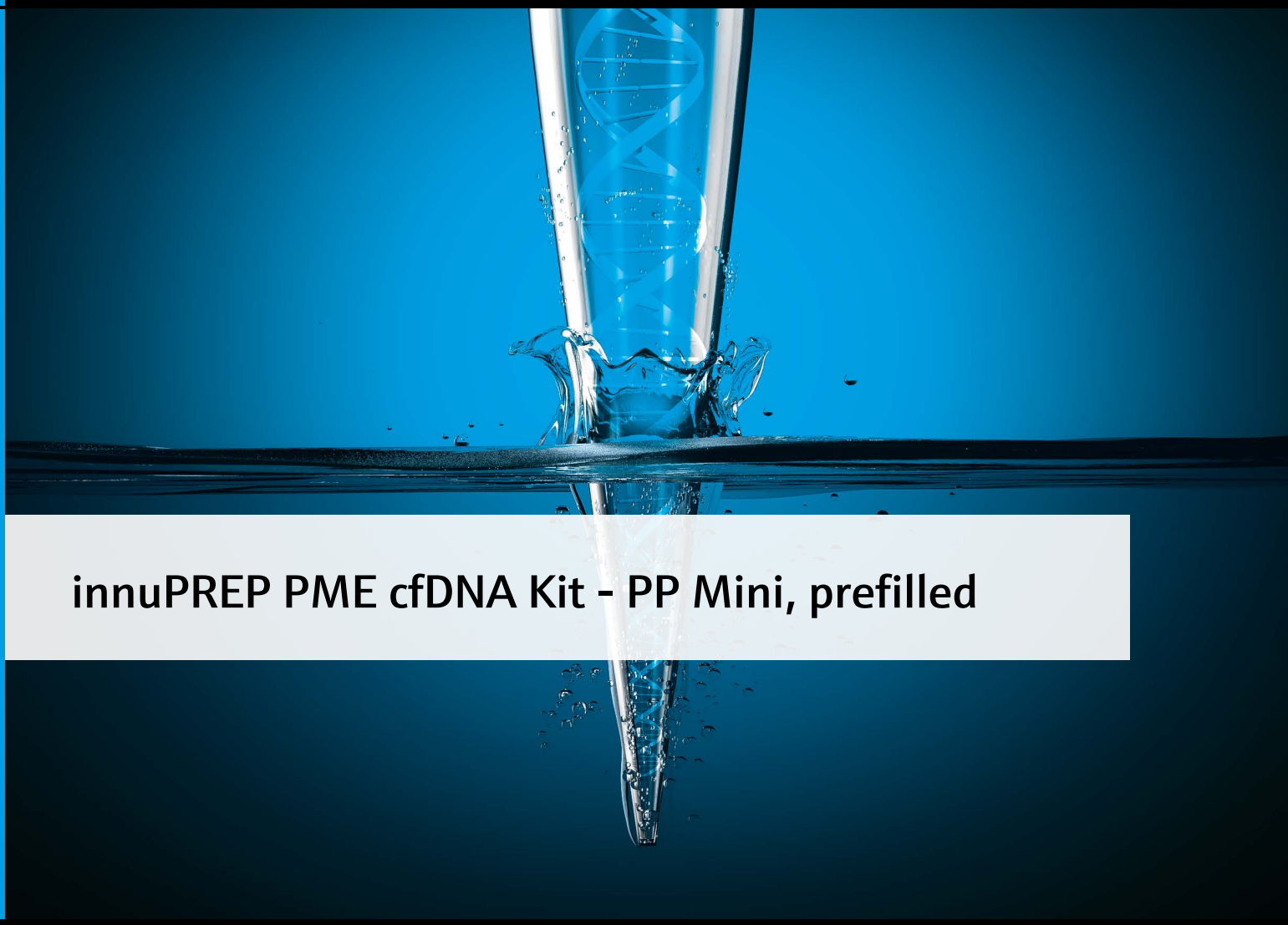
Instructions for Use

Life Science Kits & Assays



innuPREP PME cfDNA Kit - PP Mini, prefilled

Innuscreen

innovative Sensor Technology

Order No.:

845-PPA-1680016	16 reactions
845-PPA-1680096	96 reactions
845-PPB-1680016	16 reactions
845-PPB-1680096	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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Contents

1 Introduction 2
1.1 Intended use 2
1.2 Notes on the use of this manual and the kit 4
2 Safety precautions 5
3 Storage conditions 6
4 Functional testing and technical assistance 7
5 Product use and warranty 7
6 Kit components 8
6.4 Components included in the kit 8
6.5 Components not included in the kit 8
7 Product specifications 9
8 Quantification and Quality Assessment of cfDNA 9
8.1 Limitations of UV-Based Quantification 9
8.2 Recommended Analytical Methods for cfDNA Quantification and Characterization 10
8.3 Summary and Recommendations 12
9 Initial steps before starting 12
10 Protocol: Isolation of cfDNA from 2.5 to 3.5 ml plasma, liquor or cell culture supernatant 13
10.1 Preliminary steps 13
10.2 Enrichment of cfDNA 13
11 Automated extraction using PurePrep Mini 14
11.1 General filling scheme of the reagents (already prefilled) 14
11.2 Unpacking of Reagent Plate and peeling off the sealing foil 14
11.3 Prefilled DW Plate or DW Strips 15
11.4 Loading filled Deep Well Plate/Strips to the PurePrep Mini and plug in the Tip Combs 16
12 Troubleshooting 18

1 Introduction

1.1 Intended use

The **innuPREP PME cfDNA Kit – PP Mini, prefilled** has been designed for the isolation of circulating cell-free DNA (cfDNA) from 2.5 – 3.5 ml plasma, liquor & cell culture supernatant. This kit is based on new technology, called: PME – Polymer Mediated Enrichment. The procedure does not start with sample lysis, like commonly used methods or other commercially available kits.

Most 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. Due to the very low concentration of circulating cell-free DNA in plasma and liquor, these methods require large sample volumes to obtain sufficient DNA for downstream applications. As a result, these methods are very time and work consuming, labor intensive and require a lot of reagents.

In contrast to other commercially available kits, the PME method begins with the capturing of circulating cell-free DNA with a special polymer which leads to reduction of the sample volume drastically. Subsequently the captured circulating cell-free DNA in special polymer is dissolved in a special lysis buffer and then the cfDNA is extracted.

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 Reagent Plate / Strip of the kit is prefilled with almost all extraction reagents which are needed for the extraction process.

The kit is intended for use by professional users. cfDNA extracted using this Kit can be used in 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 regarding other

clinical or laboratory results. To reduce irregularities in diagnostic results, internal controls for downstream applications should be used.

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.

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 G** and **Enrichment Reagent VCR-1** at 4 °C to 8 °C.

All other components of the **innuPREP PME cfDNA Kit – PP Mini, prefilled** 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 is 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 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, prefilled** 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

6.4 Components included in the kit

	Σ 16	Σ 96
REF	845-PPA-1680016 [a] 845-PPB-1680016 [b]	845-PPA-1680096 [a] 845-PPB-1680096 [b]
SafeSeal Tube 5.0 ml	16	96
MAG Suspension G	100 µl	0.5 ml
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution
Lysis Solution RL	8 ml	50 ml
Enrichment Reagent VCR-1	1.2 ml	5 x 1.2 ml
Enrichment Reagent VCR-2	2 x 2 ml	20 ml
Reagent Plate D (PP) [a]	1	6
Reagent Strip D (PP) [b]	16	96
Tip Combs [a]	2	12
Tip Combs [b]	4	24
Manual	1	1

6.5 Components not included in the kit

- Magnetic rack for 5 ml tubes
- 1.5 ml Elution tubes
- ddH_2O for dissolving **Proteinase K**

7 Product specifications

1. **Starting material:**

- 2.5 – 3.5 ml plasma, liquor or cell culture supernatant

2. **Time for isolation:**

- Approximately 70 minutes including all steps

8 Quantification and Quality Assessment of cfDNA

cfDNA is typically present in plasma, liquor or in cell culture supernatant at very low concentrations, often in the range of several ng/ml or even lower and predominantly in short DNA fragments. Due to these characteristics, accurate quantification and quality assessment require analytical methods with high sensitivity and specificity. This chapter provides an overview of the limitations of UV-based quantification and outlines recommended approaches for accurate cfDNA measurement and quality control.

8.1 Limitations of UV-Based Quantification

- Insufficient Sensitivity of UV Spectrophotometry
  UV spectrophotometers (e.g., NanoDrop™) are widely used for nucleic acid quantification. However, they are not suitable for cfDNA analysis because the cfDNA concentration typically lies below the quantitative detection limit of UV absorbance methods. In this low concentration range, the measured signal is commonly indistinguishable from instrumental background noise, resulting in inaccurate or artificially inflated values.

- Interference from Extraction Reagents and Non-interpretability of $A_{260/280}$ and $A_{260/230}$ Ratios
  Buffers used in cfDNA extraction frequently contain compounds such as chaotropic salts, EDTA, or detergents, which absorb light at 230 nm and 260 nm. Because the concentration of these reagents often exceeds that of the target cfDNA, they significantly distort both concentration estimates and purity assessments. Furthermore, at such low nucleic acid concentrations, the purity and ratios lose their

diagnostic value; even minor background fluctuations in the instrument or reagents result in extreme ratio shifts. Consequently, these values fail to provide meaningful information regarding sample purity or extraction success.

Conclusion:
UV-based spectrophotometric methods should not be used for cfDNA quantification or purity assessment, as they do not provide reliable or interpretable results.

8.2 Recommended Analytical Methods for cfDNA Quantification and Characterization

- Fluorescence-Based Quantification Methods
  Fluorescent dye–based assays (e.g., Qubit™, Quantus™, PicoGreen® or equivalent platforms) offer highly specific and sensitive quantification of low DNA concentrations. Unlike UV-based methods, these dyes selectively bind double-stranded DNA, minimizing interference from RNA, proteins, or buffer components.

  Key Advantages:

  - Detection limits extend into the low picogram range
  - High specificity for DNA
  - Excellent reproducibility and robustness to UV-absorbing contaminants
  - Minimal sample volume required

Conclusion:
Fluorescence-based assays represent the recommended standard method for determining cfDNA concentration.

- Fragment Size Analysis via Capillary Electrophoresis
  Automated capillary electrophoresis platforms, such as the Agilent TapeStation™ or Bioanalyzer™, enable simultaneous quantification and high-resolution assessment of fragment size distributions. This information is essential for evaluating cfDNA integrity and for downstream applications such as next-generation sequencing (NGS).

Typical cfDNA profiles show a predominant peak corresponding to mono-nucleosomal fragments (approximately 160–180 bp). Deviations from this pattern, including the presence of larger genomic DNA fragments, may indicate preanalytical processing issues or cellular lysis.

Key Advantages:

- Highly sensitive concentration measurement
- Detailed resolution of fragment length distribution
- Identification of genomic DNA contamination
- Direct visualization of the characteristic cfDNA fragmentation pattern

Conclusion:
This technology represents the gold standard for cfDNA quality assessment.

- Quantification by Real-Time PCR Assays (qPCR or ddPCR)
  Real-time quantitative PCR (qPCR) and digital PCR (ddPCR) assays offer the highest sensitivity for cfDNA quantification. These methods measure amplifiable DNA within specific genomic regions, thereby providing functional insights into cfDNA integrity.

Key Advantages:

- Ultra-high sensitivity for low DNA concentrations
- Quantification of specific genomic regions
- Assessment of amplifiable, intact cfDNA
- Suitable for samples intended for PCR and NGS workflows

Conclusion:
Real-Time PCR-based assays are recommended when maximal sensitivity and functional quantification are required for high-complexity applications.

8.3 Summary and Recommendations

For reliable cfDNA analysis, only fluorescence-based, capillary electrophoresis–based, or PCR-based methods should be used. The table below summarizes the recommended approaches.

Recommended Analytical Methods for cfDNA:

Analytical Goal	Not Suitable	Recommended Method
Concentration Measurement	UV spectrophotometry (NanoDrop™)	Fluorescence-based quantification (e.g., Qubit™)
Quality and Fragment size Analysis	UV purity ratios	Capillary electrophoresis (TapeStation™, Bioanalyzer™)
Functional Integrity Assessment	UV absorbance	qPCR or ddPCR

9 Initial steps before starting

- Add the indicated amount of ddH_2O to **Proteinase K**, mix thoroughly and store as described above.

845-PPA-1680016 845-PPB-1680016	Add 0.3 ml ddH_2O to each lyophilized Proteinase K.
845-PPA-1680096 845-PPB-1680096	Add 1.5 ml ddH_2O to each lyophilized Proteinase K.

- Centrifugation steps should be carried out at room temperature.

10 Protocol: Isolation of cfDNA from 2.5 to 3.5 ml plasma, liquor or cell culture supernatant

10.1 Preliminary steps

It is important to remove residual cells and debris. We recommend a centrifugation of the sample at 5.000 × g for 20 minutes at room temperature to remove residual cells and debris.

After centrifugation transfer the whole supernatant (2.5 to 3.5 ml) into SafeSeal Tube 5.0 ml (included).

10.2 Enrichment of cfDNA

1. Add the reagents to the SafeSeal Tube containing the sample in the following order
   - **60 µl Enrichment Reagent VCR-1**,
   - **4 µl MAG Suspension G** and
   - **Enrichment Reagent 160 µl VCR-2** and mix the tube vigorously by vortexing.

NOTE
Vortex the MAG Suspension G vigorously before use!

2. Incubate the SafeSeal Tube for 20 minutes while shaking with low speed end-over-end (e.g. use a rotator).
3. Place the SafeSeal Tube into a magnetic rack to collect the magnetic beads. Leave it until the solution is clear & discard the supernatant as completely as possible (e.g. using a pipet).
4. Remove the SafeSeal Tube from the magnetic rack and add **450 µl Lysis Solution RL** and **30 µl Proteinase K.**
5. Incubate for 5 minutes and resuspend the beads completely using a pipet and rinse the residual beads from the wall.
6. Proceed with “Automated extraction using PurePrep Mini” on p. 14

11 Automated extraction using PurePrep Mini

11.1 General filling scheme of the reagents (already prefilled)

Cavity of DW Plate/Strip	Content
Cavity 1	500µl Binding Solution V
Cavity 2	800 µl Washing Solution HS
Cavity 3	800 µl Washing Solution HS
Cavity 4	800 µl Washing Solution LS
Cavity 5	empty
Cavity 6	100 µl Elution Buffer

11.2 Unpacking of Reagent Plate and peeling off the sealing foil

Reagent Plates and Reagent Strips are delivered wrapped into plastic bags for transport protection.

Carefully open the overpack of Reagent Plates/Strips by using scissors.

Afterwards gently remove the foil by peeling off.

11.3 Prefilled DW Plate or DW Strips

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1					Eluate 1	Sample 9					Eluate 9
B	Sample 2					Eluate 2	Sample 10					Eluate 10
C	Sample 3					Eluate 3	Sample 11					Eluate 11
D	Sample 4					Eluate 4	Sample 12					Eluate 12
E	Sample 5					Eluate 5	Sample 13					Eluate 13
F	Sample 6					Eluate 6	Sample 14					Eluate 14
G	Sample 7					Eluate 7	Sample 15					Eluate 15
H	Sample 8					Eluate 8	Sample 16					Eluate 16

Fig. 1: Schematic illustration of DW Plate

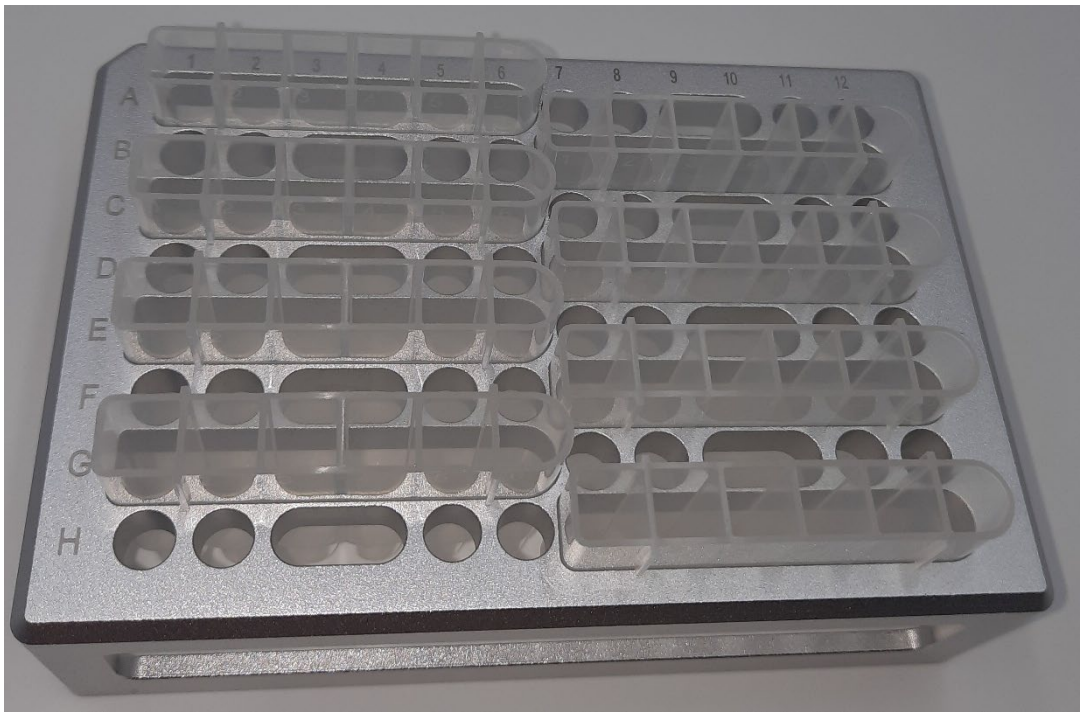


Fig. 2: Arrangement of the DW Strips in Tray (still opened)

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

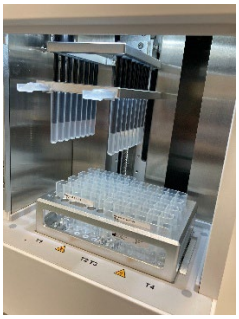
NOTE

- 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.
- When working with strip (strips), only every second tip is being used for extraction:

  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

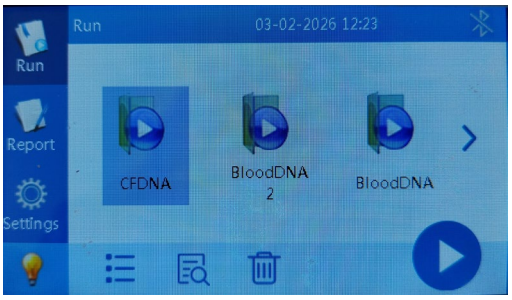
1. add 500 µl of the resuspended bead-pellet (from 10.2 step 5) to cavities 1/7 of the Plate or to cavity 1 of the strip placed in the Holder



Reagent Strips

Reagent Plate

2. Place the Plate and Strips inserted in the Holder (tray) into the PupePrep Mini with the label facing outward.
3. Insert the tip comb in the tip comb holder.
4. Select the protocol “CFDNA” and start the run.



5. After finishing the extraction protocol, the Cavities 6 and 12 contain the isolated DNA.

6. Transfer the DNA into a fresh 1.5 ml Tube.

IMPORTANT NOTE

Store the isolated DNA under adequate conditions. We recommend storing the extracted DNA for longer use at −22 °C to -18 °C.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
DNA not measurable by spectrophotometer/Poor UV purity ratios	
Concentration is below the detection limit of UV spectrophotometry	UV absorbance-based quantification (e.g., NanoDrop™) is **not suitable** for cfDNA. Refer to **Section 8 Quantification and Quality Assessment of Circulating Cell-Free DNA (cfDNA)** Use an appropriate method such as **Qubit™, TapeStation™, Bioanalyzer™, qPCR, or ddPCR**.
No extracted DNA	
No addition of Binding Solution	Make sure that Binding Solution is added.
Low concentration of extracted DNA	
Too much Elution Buffer used	Elute the DNA in a lower volume of Elution Buffer (min. 80 µl).
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 or Elution tubes

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