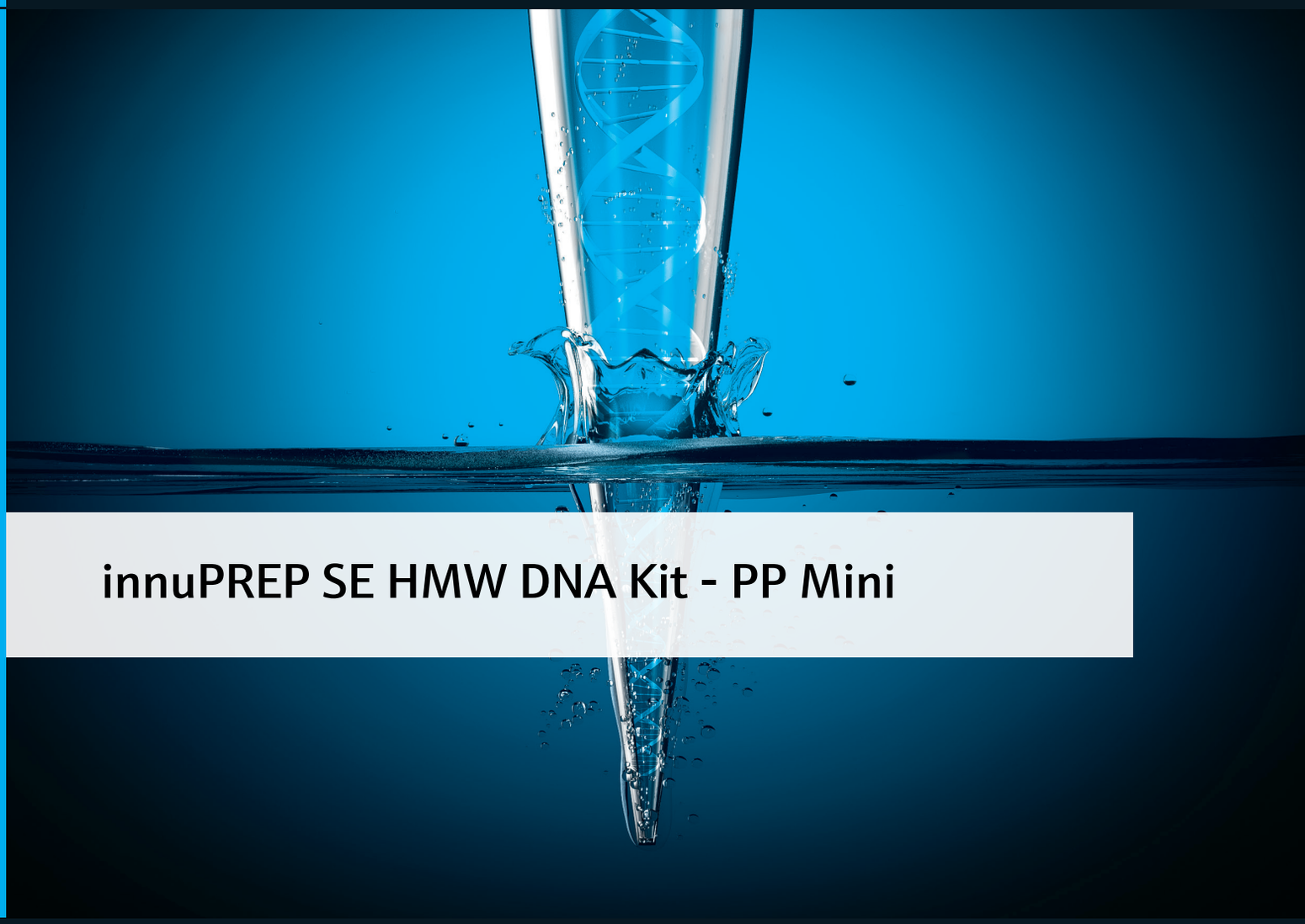
Instructions for Use
Life Science Kits & Assays



innuPREP SE HMW DNA Kit - PP Mini

Innuscreen GmbH

Order No.:

845-PSS-4216016	16 reactions
845-PSP-4216016	16 reactions
845-PSS-4216096	96 reactions
845-PSP-4216096	96 reactions

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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

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1 Introduction

1.1 Intended use

The **innuPREP SE HMW DNA Kit – PP Mini** has been designed for automated isolation of high molecular weight DNA (HMW) from bacteria, yeasts, tissue samples and rodent tails, eucaryotic cells and blood cells.

The kit is based on the patented SmartExtraction Technology using Smart Modified Surfaces invented by IST Innuscreen GmbH.

The extraction process is based on adsorption of the genomic DNA to Smart Modified Surfaces and it needs no magnetic particles for DNA binding. That means, the DNA binds direct on the surface of the modified Pureprep Mini Tip Combs. After washing, the genomic DNA is eluted from the Smart Modified Surfaces and is ready for use in subsequent downstream applications.

The whole extraction process just needs simple mixing up and down of the modified Tip Combs. The process is very fast and gives no limitation regarding the binding capacity. So, the kit is optimized to get a maximum of yield and quality. Further, because of the extraction technology the DNA is from excellent quality and is high molecular weight DNA.

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 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** at 4 °C to 8 °C and **RNase A** at -22 to -18 °C.

All other components of **the innuPREP SE HMW DNA 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.

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 SE HMW 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-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 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 16	Σ 96
REF	845-PSS-4216016 [a] 845-PSP-4216016 [b]	845-PSS-4216096 [a] 845-PSP-4216096 [b]
Ery Lysis Solution A (conc.)	2 x 11 ml	2 x 60 ml
Ery Lysis Solution B (conc.)	10 ml	60 ml
Lysis Solution CBV	10 ml	50 ml
Proteinase K	for 1 x 1.5 ml working solution	for 4 x 1.5 ml working solution
RNase A	2 x 60 µl	600 µl
Binding Optimizer	1 ml	5 ml
Washing Solution MS (conc.)	9 ml	54 ml
Washing Solution ER	17 ml	85 ml
Elution Buffer	7 ml	50 ml
Modified Tip Comb [a]	4	24
Modified Tip Comb [b]	2	12
DW Strip [a]	16	96
KF96 DW Plate [b]	1	6
Manual	1	1

6.2 Components not included in the kit

- 96 %–99.8 % ethanol (molecular biology grade, undenatured)
- 70 % ethanol
- Isopropanol
- ddH_2O; ultrapure for dissolving Proteinase K

6.3 Components needed for isolation from bacteria (not included)

- Lysozyme (stock solution 10 mg/ml (400 U/µl))
- Mutanolysin (stock solution 0.4 U/µl)
- Lysostaphin (stock solution 0.4 U/µl)
- 1 x TE-Buffer

Alternatively:

- innuPREP Bacteria Lysis Booster (845-KA-1000050, 50 rxn, IST Innuscreen GmbH)

6.4 Components needed for isolation from yeasts (not included)

- Yeast Digest Buffer (50 mM KH_2PO_4, 10 mM DTT, pH 7.5)
- Lyticase (stock solution 10 U/µl)

7 Product specifications

1. Starting material:

- Tissue samples (5 mg – 40 mg)
- Rodent tails (0.1 - 0.5 cm; max. 50 mg)
- Bacteria / Yeast cell pellets (1×10^8 – 1×10^{12} cells)
- Eukaryotic cells (1×10^6 – 1×10^7)
- Whole blood for collecting of PBMC's (1 – 3 ml; depends on amounts of PBMC's use not more blood as equivalents to 1×10^7 PBMC's)

2. Time for automated extraction:

- 43 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-PSS/PSP-4216091	Add 1.5 ml ddH_2O to lyophilized Proteinase K.
845-PSS/PSP-4216096	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Add the indicated amount of ethanol to **Washing Solution MS (conc.)** and mix thoroughly. Always keep the bottles firmly closed!

845-PSS/PSP-4216016	Add 21 ml ethanol to 9 ml **Washing Solution MS**.
845-PSS/PSP-4216096	Add 126 ml ethanol to 54 ml **Washing Solution MS**.

- Use appropriate bottles and add the indicated volumes of **Ery Lysis Solution A (conc.)** to ddH_2O and mix thoroughly. Always keep the bottles firmly closed!

845- PSS/PSP-4216016	Add 11 ml **Ery Lysis Solution A** to 99 ml ddH_2O.
845- PSS/PSP -4216096	Add 60 ml **Ery Lysis Solution A** to 540 ml ddH_2O.

- Use appropriate bottles and add the indicated volumes of **Ery Lysis Solution B (conc.)** to ddH_2O and mix thoroughly. Always keep the bottles firmly closed!

845- PSS/PSP -4216016	Add 10 ml **Ery Lysis Solution B** to 90 ml ddH_2O.
845- PSS/PSP -4216096	Add 60 ml **Ery Lysis Solution B** to 540 ml ddH_2O.

9 Sample preparation

9.1 Sample preparation of tissue samples and rodent tails

1. Cut the sample into small pieces and transfer them into a 1.5 ml tube.

2. Add **400 µl Lysis Solution CBV** and **30 µl Proteinase K** and vortex the tube shortly.

NOTE
Optionally add to each sample 5 µl RNase A (10mg/ml).

3. Place the tube into a Thermoshaker and incubate at 1.000 rpm and 55°C to lyse the sample material. Lysis time depends on kind of tissue sample. In generally lysis can be stopped if most of the material is lysed (e.g. 0.5 cm mouse tail: approx. 90 minutes). After lysis centrifuge the tube at maximum speed for 5 minutes to pellet down the unlysed material. Carefully transfer the supernatant into the first cavity of the DW Plate or the DW Strip.

   Proceed with “Automated extraction using PurePrep Mini” on p.15.

9.2 Sample preparation of bacteria & yeast cells

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 min at 3,000 x g) and discard the supernatant.

2. Resuspend the bacterial cell pellet in 200 µl TE Buffer and the yeast cell pellet in 200 µl Yeast Digest Buffer (→ see "Components needed for isolation from yeasts", p. 8). in a 1.5 ml tube and add the enzymes according to the table below or follow the instruction of the product innuPREP Bacteria Lysis Booster:

	Enzyme	Volume
Gram-negative bacteria	Lysozyme: 10 mg/ml (400 U/µl)	20 µl
Staphylococcus spp.	Lysostaphin: 0.4 U/µl	10 µl
Gram-positive bacteria	Lysozyme: 10 mg/ml (400 U/µl) + Mutanolysin: 0.4 U/µl	20 µl + 5µl
Yeast	Lyticase: 10 U/µl	10 µl

3. Incubate at 37 °C for 30 min under continuous shaking.

NOTE
Optionally add 5 µl RNase A (10mg/ml) to each sample.

4. Add **200 µl Lysis Solution CBV** and **20 µl Proteinase K** and vortex the tube shortly.

5. Place the tube into a Thermoshaker and incubate at 1.000 rpm and 55°C for 30 minutes. Carefully transfer the supernatant into the first cavity of the DW Plate or the DW Strip.

Proceed with "Automated extraction using PurePrep Mini" on p.15.

9.3 Sample preparation from 1-3 ml whole blood

1. Dispense Ery Lysis Solution A according to the volume of whole blood sample (see table below) into a 15 ml tube.

Whole blood volume	Volume of Ery Lysis Solution A
1.0 ml	5.0 ml
2.0 ml	8.0 ml
3.0 ml	10.0 ml

2. Add 1 – 3 ml whole blood into the prepared 15 ml tube and mix by inverting 6 times.
3. Incubate 5–10 minutes at room temperature. Invert at least once during incubation time.

NOTE
For fresh blood (collected within 1–6 h before starting the extraction) increase incubation time to 10 minutes to ensure complete lysis of red blood cells. Do not use more blood than correspond to 1 x 10^7 PBMC's!

4. Centrifuge for 3 minutes at 1,500 x g to pellet the PBMC.
5. **Carefully** discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet!

6. Add **5 ml Ery Lysis Solution B** to the PBMC pellet and vortex shortly or shake the tube vigorously to resuspend the cell pellet completely.
7. Centrifuge for 3 minutes at 1,500 x g to pellet the PBMC.
8. Carefully discard the supernatant by pipetting or pouring.

NOTE
Do not discard the PBMC pellet! Use a paper towel to remove residual liquid as much as possible!

9. Add **130 µl Ery Lysis Solution B** to the cell pellet and resuspend the pellet as much as possible by pipetting up and down. Adjust the pipet to 200 µl (not more).

10. Transfer max. 220 µl of resuspended PBMC into a 1.5 ml tube.
11. Add **200 µl Lysis Solution CBV** and **30 µl Proteinase K** (for 3.0 ml blood sample use 50 µl Proteinase K).

NOTE
Optionally add 5 µl RNase A (10mg/ml) to each sample.

12. Place the tube into a Thermoshaker and incubate at 1.000 rpm and 55°C for 30 minutes. Carefully transfer the supernatant into the first cavity of the DW Plate or the DW Strip.

    Proceed with “Automated extraction using PurePrep Mini” on p.15.

9.4 Sample preparation from eucaryotic cells (1×10^6 – 1×10^7 cells)

1. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 3 minutes at 1,500 x g) and discard the supernatant as much as possible.
2. Add **130 µl Ery Lysis Solution B** to the cell pellet and resuspend the pellet as much as possible by pipetting up and down. Adjust the pipet to 200 µl (not more).
3. Transfer max. 220 µl of resuspended cells and transfer the cells into a 1.5 ml tube.
4. Add **200 µl Lysis Solution CBV** and **40 µl Proteinase K** and vortex shortly.

NOTE
Optionally add 5 µl RNase A (10mg/ml) to each sample.

5. Place the tube into a Thermoshaker and incubate at 1.000 rpm and 55°C for 30 minutes. Carefully transfer the supernatant into the first cavity of the DW Plate or the DW Strip.

   Proceed with “Automated extraction using PurePrep Mini” on p.15.

10 Automated extraction using PurePrep Mini

10.1 Prefilling of the DW Plate or the 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

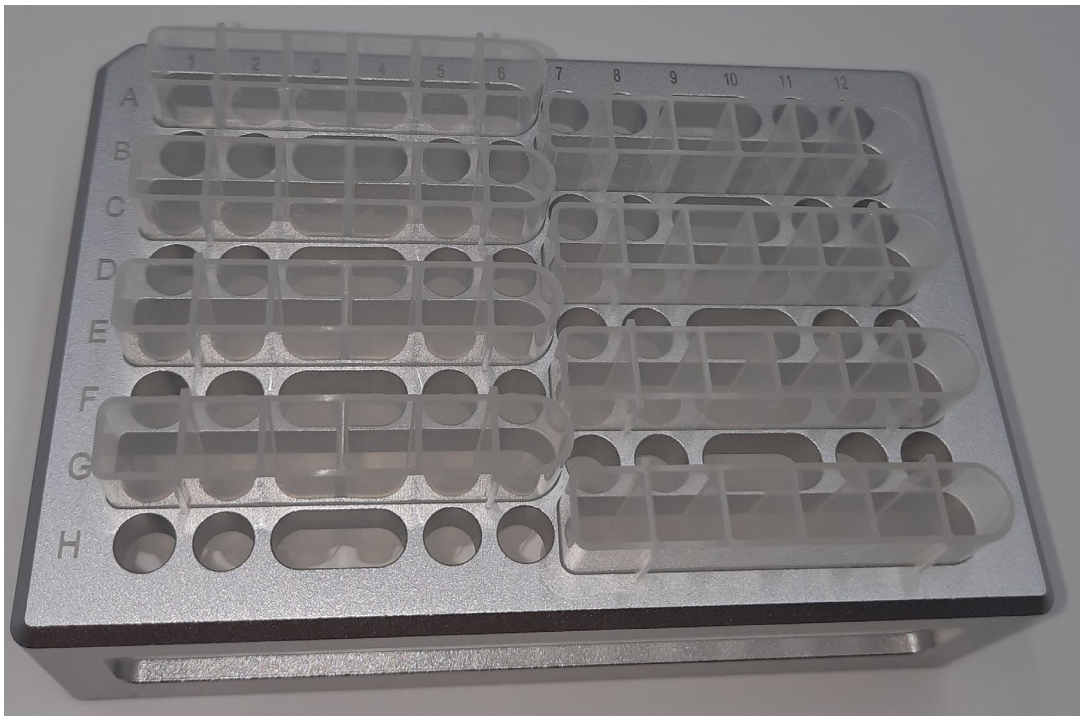

Fig. 2: Arrangement of the DW Strips in Tray

Cavity of DW Plate/Strip	Content
Cavity 1	Lysed sample + 400 µl Isopropanol + 50 µl Binding Optimizer
Cavity 2	800 µl Washing Solution MS
Cavity 3	800 µl Washing Solution MS
Cavity 4	800 µl 70% Ethanol
Cavity 5	800 µl Washing Solution ER
Cavity 6	200 µl – 400 µl Elution Buffer (volume depends on starting material and expected yield)

10.2 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 modified 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

   "PPMiniSE2" and start the run.
2. After finishing the extraction protocol, the Cavity 6 contains the isolated HMW DNA.
3. Transfer the DNA into a fresh 1.5 ml Tube using a wide-bore tip or a cut-off pipette tip.

IMPORTANT NOTE HIGH MOLECULAR WEIGHT DNA

The HMW DNA might be very viscous. The dissolving step is crucial for successful extraction and for a maximum of yield. If the DNA content is too high, increase the amount of Elution Buffer.

HMW gDNA needs time to relax. It is generally not recommended to work with freshly eluted DNA unless significant effort is made to ensure even DNA resuspension. Letting a sample relax overnight or for several days facilitates homogenization. If possible, it is recommended that HMW DNA is extracted several days or a week prior to being needed for downstream application.

If you do not need high molecular weight DNA you can shear the DNA e.g. by using ultrasound or by passing the eluate through a needle or a shredder spin filter unit.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted DNA	
Preparation without Binding Optimizer and Isopropanol	Pay special attention that Binding Optimizer and Isopropanol was added to the lysed sample!
High viscosity of extracted DNA / Inhomogeneous DNA solution	
Insufficient amount of Elution Buffer	Refer to the note of HMW DNA and the DNA with a higher volume of RNase-free Water
Relax time to short	Refer to the note of HMW DNA and let the DNA relax overnight at 2-8°C
Degraded or sheared DNA	
Old material insufficient	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion

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

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