



Application Note – innuPREP SE HMW DNA Kit –PP Mini

Automated extraction of DNA from different starting materials

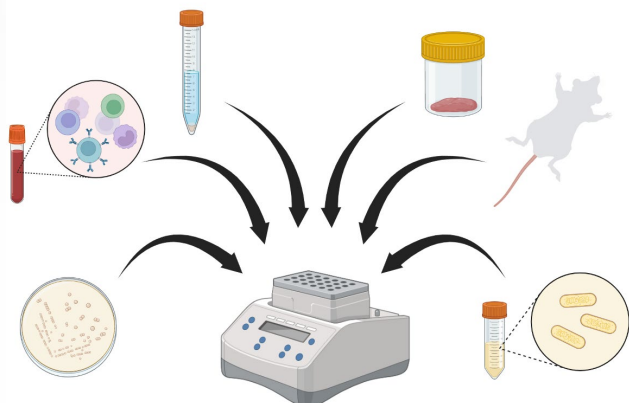
Short Product Description

The innuPREP SE HMW DNA Kit – PP Mini has been designed for automated extraction of high molecular weight (HMW) DNA from different starting materials, among which include eukaryotic cells, bacterial and yeast cultures, tissue samples, rodent tails and whole blood. The kit is based on the patented SmartExtraction Technology using Smart Modified Surfaces invented by IST Innuscreen GmbH. The extraction method relies on the adsorption of genomic DNA (gDNA) to Smart Modified Surfaces of the modified PurePrep Tip Comb and required no magnetic particles for DNA binding. After washing, gDNA is eluted from the Smart Modified Surfaces and can be used for several downstream applications (NGS, qPCR, ddPCR, etc.)

The PurePrep Mini is a remarkably compact benchtop device specialized in DNA and RNA extraction and purification. Ideally for small-scale laboratories. The PurePrep Mini allows simultaneous processing of up to 16 samples.

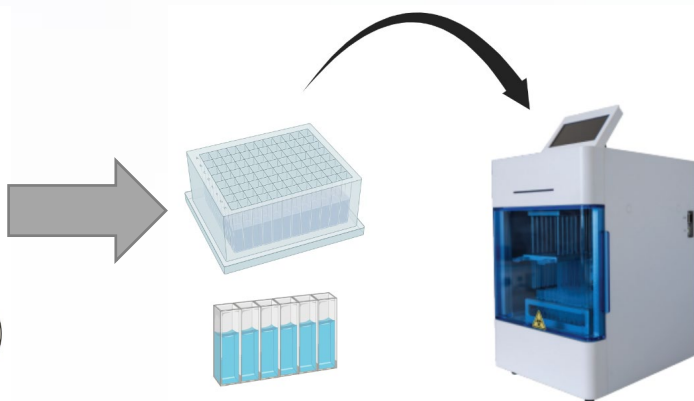
Workflow: Automated DNA extraction from different starting materials

Part 1: Manual Step External lysis



Addition of Lysis Buffer and Proteinase K & incubation

Part 2: Automated Extraction



Addition of lysed samples to deep-well plate or strips and automated extraction run

PurePrep Mini extraction and purification steps:

- Binding (10 min)
- Washing (1 min)
- Elution (30 min)

Examples of Application

I-A. Extraction of DNA from mouse tails

Starting material: 20 mg mouse tail / 40 mg mouse tail

Addition of Lysis Solution CBV and Proteinase K and lysis at 55°C for 90 minutes using a thermoshaker. Automated DNA extraction was performed by transferring the lysate into a deep-well plate. After addition of Binding Buffers the automated extraction takes place. After extraction the DNA were transferred to a clean 1.5 ml tube.

I-B. Application results: analysis of HMW DNA from mouse tail

To visualize and confirm DNA from the extracted samples, a 0.7% agarose gel electrophoresis was performed. 10 μ l DNA per sample was run on the gel at 100 V between 75 to 90 minutes. Results of agarose gel electrophoresis showed a successful extraction of DNA using the PurePrep Mini system (Figure 1A). To determine the DNA yield and quality, 1 μ l DNA was used on Agilent 4150 TapeStation System (Agilent) and used for spectrophotometric measurements. The results indicate that majority of the DNA is longer than 60,000 bp and shows a high integrity. The DNA yield is very high and the ratios between 260/280 and 260/230 are excellent.

Table 1. Spectrophotometric measurements of HMW DNA extracted from mouse tails.

Sample name	DNA yield (μ g)	A _{260/280}	A _{260/230}
Mouse Tail_1 (20 mg)	9	1.9	1.9
Mouse Tail_2 (40 mg)	19	1.9	1.9

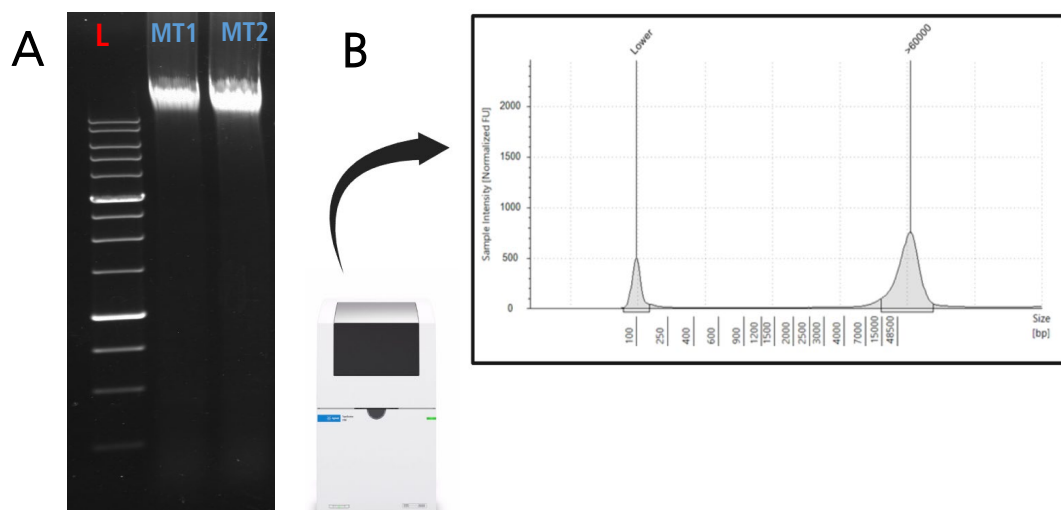


Figure 1. (A). Gel electrophoresis photo; L: Ladder; MT1: mouse tail sample 1; MT2: mouse tail sample 2. (B) Agilent 4150 TapeStation analysis

II-A. Extraction of DNA from mouse lung tissues

Starting material: 7 mg lung sample / 15 mg lung sample

Addition of Lysis Solution CBV and Proteinase K and lysis at 55°C for 60 minutes using a thermoshaker. Automated DNA extraction was performed by transferring the lysate into a deep-well plate. After addition of Binding Buffers the automated extraction takes place. After extraction the DNA were transferred to a clean 1.5 ml tube.

II-B. Application results: analysis of HMW DNA from mouse lung tissues

To visualize and confirm DNA from the extracted samples, a 0.7% agarose gel electrophoresis was performed. 10 μ l DNA per sample was run on the gel at 100 V between 75 to 90 minutes. Results of agarose gel electrophoresis showed a successful extraction of DNA using the PurePrep Mini system (Figure 1A). To determine the DNA yield and quality, 1 μ l DNA was used on Agilent 4150 TapeStation System (Agilent) and used for spectrophotometric measurements. The results indicate that majority of the DNA is longer than 60,000 bp and shows a high integrity. The DNA yield is very high and the ratios between 260/280 and 260/230 are excellent (Figure 1B).

Table 2. Spectrophotometric measurements of HMW DNA extracted from mouse lung tissues.

Sample name	DNA yield (μ g)	$A_{260/280}$	$A_{260/230}$
Mouse Lung_1 (7 mg)	11	1.8	2.0
Mouse Lung_2 (15 mg)	25	1.9	1.9

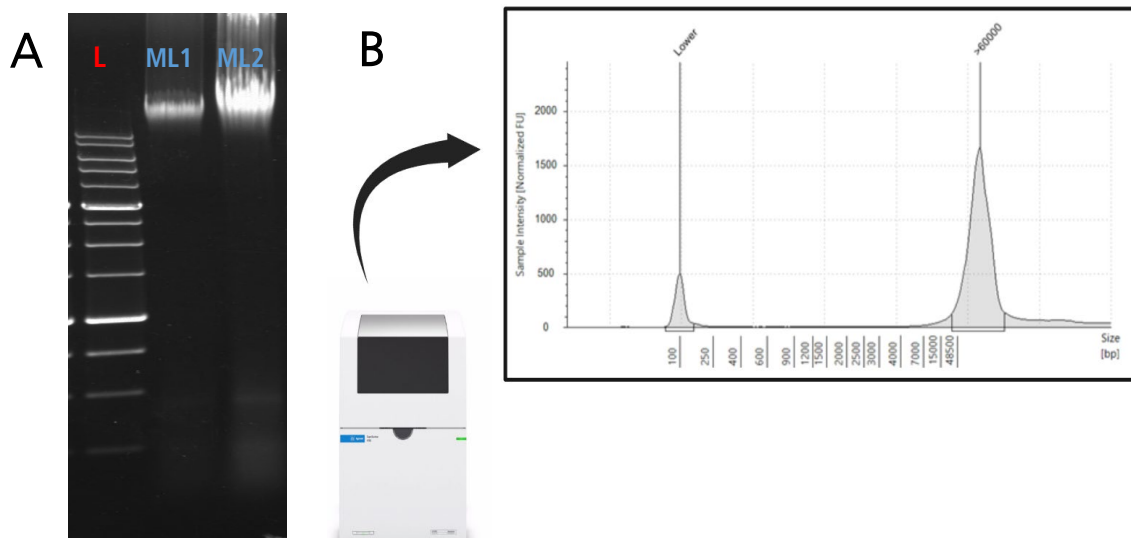


Figure 2. (A). Gel electrophoresis photo; L: Ladder; MT1: mouse lung sample 1; MT2: mouse lung sample 2. (B) Agilent 4150 TapeStation analysis

III-A. Extraction of DNA from whole blood samples

Starting material: 2 ml whole blood (3 samples)

Lysis of erythrocytes and pelleting of PBMC's. Resuspension of PBMC pellet and addition of Lysis Solution CBV and Proteinase K and lysis at 55°C for 30 minutes using a thermoshaker. Automated DNA extraction was performed by transferring the lysate into a deep-well plate. After addition of Binding Buffers the automated extraction takes place. Samples were eluted at 200 µl, 300 µl, and 400 µl, respectively, in elution buffer and transferred to clean 1.5 ml tubes.

III-B. Application results: analysis of HMW DNA from whole blood samples

To determine the DNA yield and quality, 1 µl DNA was used on Agilent 4150 TapeStation System (Agilent) and used for spectrophotometric measurements. The results indicate that majority of the DNA is longer than 60,000 bp and shows a high integrity. The DNA yield is very high and the ratios between 260/280 and 260/230 are excellent (Figure 1B). The DNA concentration fluctuates depending on elution buffer volume.

Table 3. Spectrophotometric measurements of HMW DNA extracted from human whole blood.

Sample name	Concentration (ng/µl)	DNA yield (µg)	A _{260/280}	A _{260/230}
Whole Blood_1 (EV 200 µl)	201	40.2	1.9	2.2
Whole blood_2 (EV 300 µl)	136	40.8	1.9	2.2
Whole Blood_3 (EV 400µl)	117	46.8	1.9	2.2

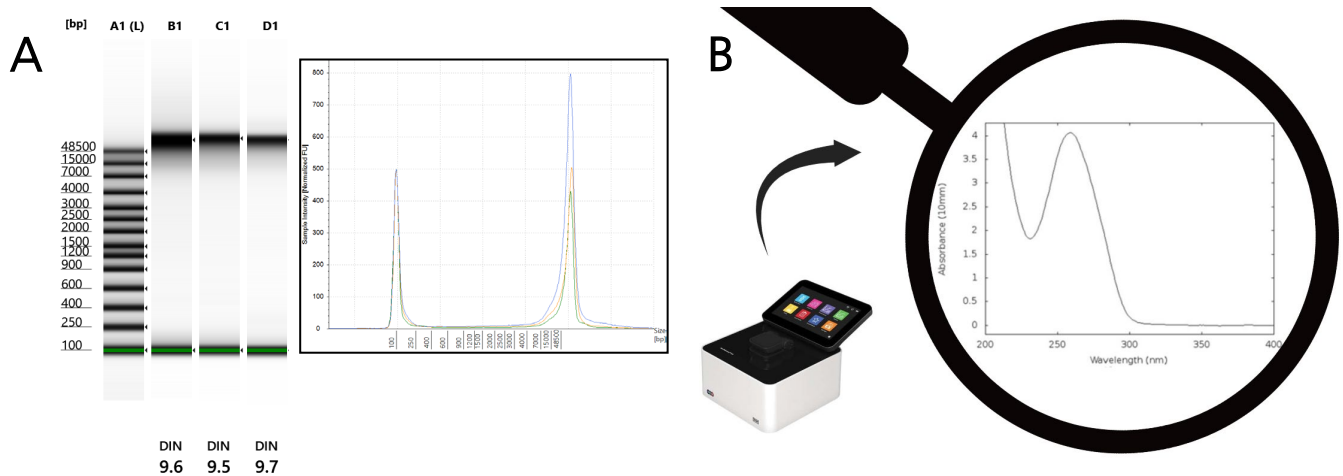


Figure 3. (A) Agilent 4150 TapeStation analysis (B) Spectrophotometrical analysis

Conclusion

The results show that DNA was successful extracted from different starting materials using the PurePrep Mini system. Based on the data from mouse tail and mouse lung tissue samples, the use of more starting materials also lead to higher DNA yield in general. Data from whole blood samples showed that the use of less elution volume may lead to higher DNA concentration, but a generally similar DNA yield. The extracted HMW DNA is from excellent quality. The whole extraction process after lysis needs only less than 45 minutes.

Order No.:	Plate /Strip
845-PSS-4296016	16 DW Strip
845-PSP-4296016	1 DW Plate
845-PSS-4296096	96 DW Strip
845-PSP-4296096	6 DW Plate