



DNA Extraction Kit and
Liquid Handling Station

SmartExtraction LHS-Kit

Protocol for HMW DNA isolation from cell pellets

Overview

The SmartExtraction LHS-Kit has been designed for automated isolation of high molecular weight DNA (HMW) from bacteria, yeasts, tissue samples and rodent tails. The kit is based on the 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.

1. Short Description of Application

Automated isolation of HMW DNA from eight cell pellet samples (taken from 2 ml whole blood per sample) was performed using the Liquid Handling Station from BRAND. The use of lysis chemistry, HMW DNA binding, and extraction is based on a technology developed by IST Innuscreen. Isolated DNA samples were analyzed and applied to different molecular experiments to determine a successful automated DNA extraction.

2. Short Protocol

The protocol begins by preparation of cell pellets from eukaryotic cells via a combination of chemical lysis and centrifugation, followed by resuspension on a buffer. Automated DNA extraction soon follows in a deep-well plate where lysed cell pellets are pipetted in one well and added with specific binding chemistry. A series of washing steps are performed using optimized washing chemistry in separate wells. Lastly, HMW DNA is eluted on a different well on an elution buffer.

3. Data Results

A) Agarose Gel Photo

To visualize and confirm HMW DNA from the eight cell pellet samples, an 0.8% agarose gel electrophoresis was performed following DNA extraction. A total of 10 µl DNA per sample was run on the gel at 120 V for 30 to 40 minutes. Agarose gel image and visualization was done using UVP GelStudio PLUS (Analytik Jena GmbH). Results showed a successful extraction of HMW DNA (Figure 1).

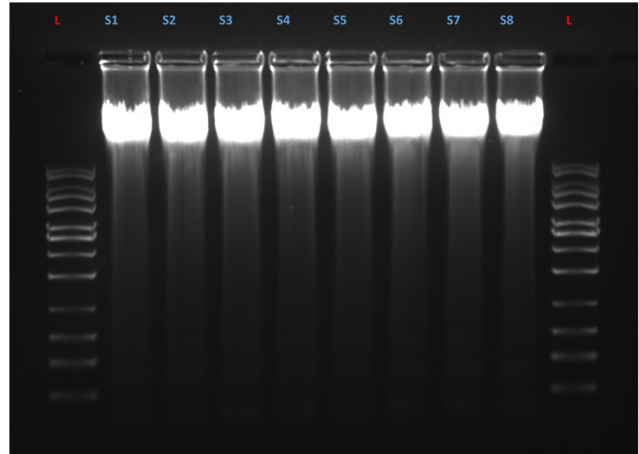


Figure 1. Gel electrophoresis photo of the eight cell pellet samples. Bright bands of high molecular weight indicated a successful DNA extraction from the eight cell pellets. L, Ladder; S1 to S8, cell pellet samples one to eight.

B) Nanophotometric Data and UV-CIS Curve

To assess DNA purity and concentration, a nanospectrophotometric measurements were performed following DNA extraction using NanoPhotometer N60® (Implen GmbH).

Sample	Concentration (ng/µl)	Yield (µg)	A _{260/280}	A _{260/230}
Sample_1	301,85	60,37	1,833	2,205
Sample_2	313,05	62,61	1,831	2,208
Sample_3	309,00	61,80	1,830	2,214
Sample_4	283,75	56,75	1,837	2,189
Sample_5	303,70	60,74	1,832	2,202
Sample_6	300,70	60,14	1,835	2,208
Sample_7	295,20	59,04	1,806	2,147
Sample_8	301,80	60,36	1,830	2,190

Table 1. Spectrophotometric measurements of cell pellet DNA. Data showed all eight cell DNA samples have a high concentration and yield along with good absorbance ratios. No contaminants were also detected in the measurements.

A total of 1 µl DNA per sample was measured and the readings were recorded. Results showed cell pellet DNA of high concentration and yield and with no contamination detected (Table 1; Figure 2).

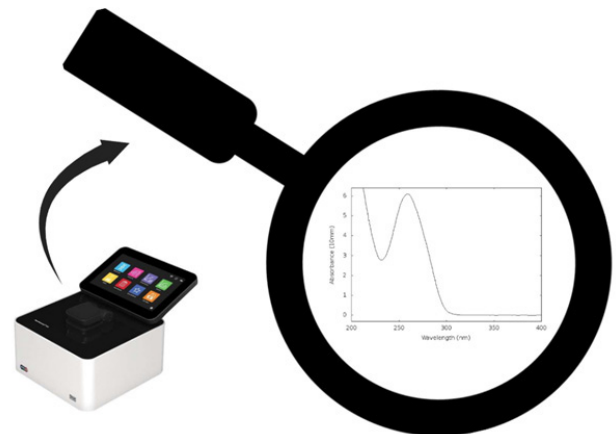


Figure 2. Visualization of UV-CIS measurement taken using the NanoPhotometer N60® in one of the cell pellet samples. Results showed a good absorbance ratio of the representative cell pellet DNA.

C) Downstream Application (RT-PCR)

To assess a successful amplification of extracted cell pellet DNA and to determine whether extracted DNA is suitable for downstream applications, an RT-PCR analysis was performed. In brief, a total of 1 µl HMW DNA was quantified using innuDRY

qRT-PCR MasterMix Probe following manufacturer's instruction. Samples were analyzed in duplicate and RT-PCR reaction was run on CFX Connect Real-Time PCR Detection System (Bio Rad). RT-PCR results indicated a successful amplification of extracted DNA and with no possible inhibitory products detected.

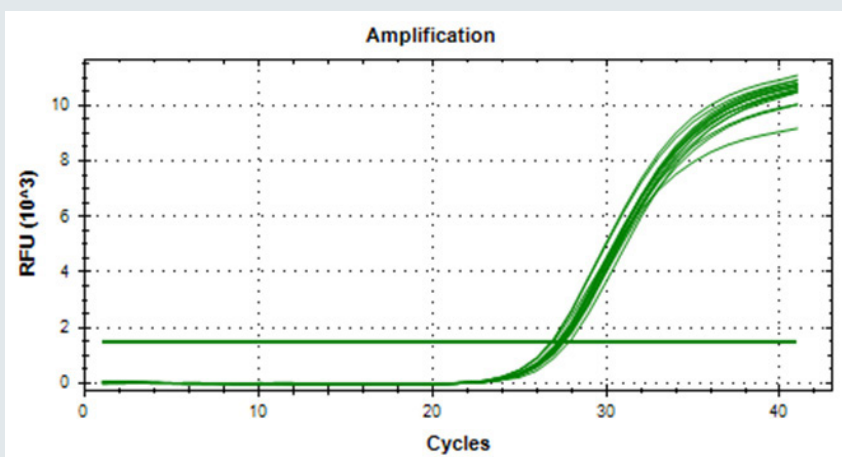


Figure 3. Successful amplification of extracted cell pellet DNA using the Liquid Handling Station. Results showed all samples were amplified in a similar rate and without any inhibitory products present.

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