DNA Extraction Kit and
Liquid Handling Station

SmartExtraction LHS-Kit

Protocol for HMW DNA isolation from mammalian tissues

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 mouse tail samples (0.5 cm or between 45 – 55 mg 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 lysed samples from mouse tails via cutting samples into small pieces, addition of lysis chemistry, and resuspension on a buffer. Automated DNA extraction soon follows in a deep-well plate where lysed mouse tail samples are pipetted in one well and added with specific binding chemistry. A series of washing steps are then 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 mouse tail 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 taken from mouse tails (Figure 1). Fuzzy bands of low molecular weight indicate the presence of mRNA in the samples, which are typical in mammalian cell samples and can be remedied by the addition of RNAse after lysis steps.

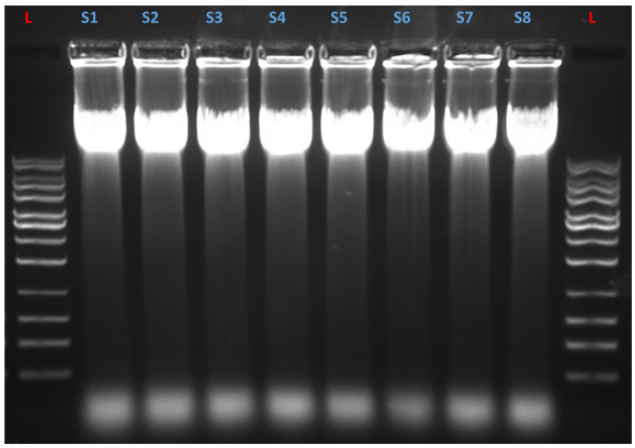

Figure 1. Gel electrophoresis photo of the eight mouse tail samples. Bright bands of high molecular weight indicated a successful DNA extraction from the eight mouse tails while fuzzy bands of low molecular weight indicate the presence of mRNA in the samples. L, Ladder; S1 to S8, mouse tail samples one 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).

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

Sample	Concentration (ng/µl)	Yield (µg)	$A_{260/280}$	$A_{260/230}$
Sample_1	346,95	69,39	1,833	2,021
Sample_2	315,45	63,09	1,881	2,033
Sample_3	312,35	62,47	1,882	2,021
Sample_4	346,55	69,31	1,878	2,039
Sample_5	350,05	70,01	1,875	2,043
Sample_6	347,95	69,59	1,883	2,050
Sample_7	352,20	70,44	1,884	2,039
Sample_8	361,30	72,26	1,879	2,027

Table 1. Spectrophotometric measurements of mouse tail 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.

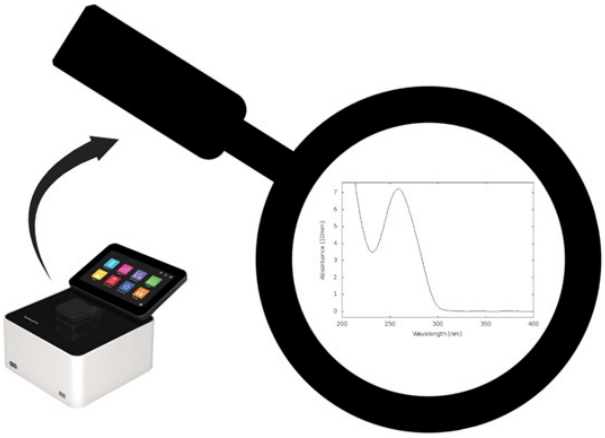

Figure 2. Visualization of UV-CIS measurement taken using the NanoPhotometer N60® in one of the mouse tail samples. Results showed a good absorbance ratio of the representative mouse tail DNA.

C) Downstream Application (RT-PCR)

To assess a successful amplification of extracted mouse tail 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 without any inhibitory products detected.

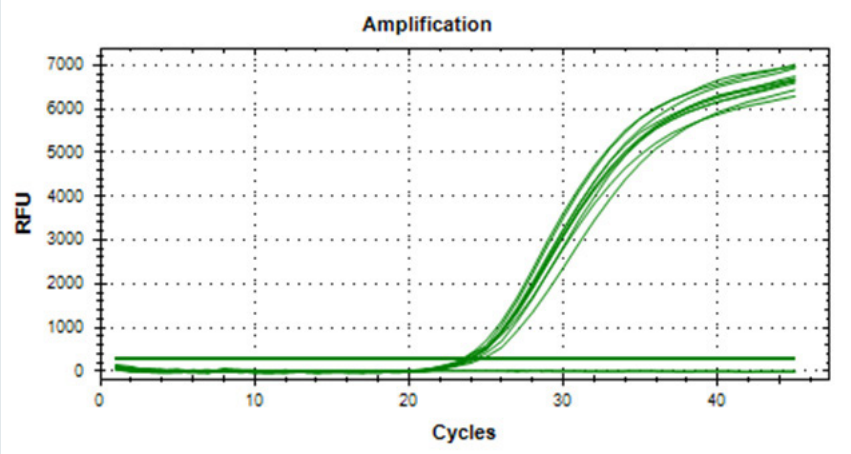

Figure 3. Successful amplification of extracted cell pellet DNA using the Liquid Handling Station. Results showed all samples were amplified in a similar rate and without any inhibitory products present.

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