Biomatrica THE BIOSTABILITY COMPANY

DNAgard Saliva HT

DNAgard® Saliva *HT:* DNA Purification Using NucleoMag® Technology

INTRODUCTION

Salivary DNA is increasingly popular for genetic analysis because saliva collection is non-invasive. DNAgard® Saliva *HT* is designed for safe collection and automated processing of salivary DNA samples. This technical bulletin provides evidence that stabilized DNA can be purified using the NucleoMag® extraction technology from Machrey-Nagel with the KingFisher™ platform from Thermo Fisher.

MATERIALS AND METHODS

Saliva Collection and Storage

Saliva samples from donors were collected into conical tubes. Samples from each individual donor were transferred into DNAgard® Saliva *HT* (DGS-*HT*) and/or Competitor O's devices and processed per the manufacturer instructions. Samples were tested in triplicate.

DNA Purification

DNA was extracted from saliva samples using the NucleoMag® Blood 200 Kit (Machrey Nagel). Samples were processed in KingFisher™ Flex Microtiter Deepwell 96 plates and 96 well Standard Elution Plates for the KingFisher™ system using the KingFisher™ Flex Magnetic Particle Processor (Thermo Fisher).

Prior to the start of the purification, four deep well plates were prepared. 200 μL of stabilized saliva, 300 μL of Binding Buffer MBL2, 25 μL of NucleoMag® B-Beads, 20 μL of 5 mg/mL Proteinase K solution, and 80 μL of water were added to each well of the Sample plate. 1000 μL of the NucleoMag Wash Buffer MBL3 was added to each well of the Wash1_1 and the Wash1_2 plates. 1000 μL of 80% Ethanol was added to each well of the Wash 2 plate. Additionally, a standard elution plate (Elution) had 200 μL of Elution Buffer MBL5 added to each well.

Table 1: Protocol for NucleoMag® Blood purification using the KingFisher™ system

Step	Plate	Step Action	Step Description	Time (min)	Elapsed Time (min)	Speed	Range	Temperature	Machrey Nagel Solution	Solution Volume (μL)	Well Volume (μL)
1	Sample	Bind	Water	NA	NA	NA	Manual Addition	Room Temperature	N/A	100	100
			Sample	NA	NA	NA	Manual Addition	Room Temperature	N/A	200	300
			Bind Mix Loop 5X	0.5	2.5	Fast	Half mix	Room Temperature	300 μL of MBL2 and 25 μL of beads	325	625
				1.0	7.5	Fast	Full volume	Room Temperature			
				0.5	10	Fast	Half Mix	Room Temperature			
			Collect Beads Loop 3x	0.5	12.5	Fast	Half mix	Room Temperature			
2	Wash 1_1	Wash 1	Release beads	0.5	13.0	Fast	Bottom mix	Room Temperature	MBL3	1000	1000
			Wash Loop 3x	0.5	14.5	Fast	Half mix	Room Temperature			
				1.0	17.5	Fast	Full volume	Room Temperature			
				0.5	19.0	Fast	Half mix	Room Temperature			
			Collect Beads 3x	0.5	20.5	Slow	Full volume	Room Temperature			
3	Wash 1_2	Wash 2	Release beads	0.5	21.0	Fast	Bottom mix	Room Temperature	MBL3	1000	1000
			Wash Loop 3x	0.5	22.0	Fast	Bottom mix	Room Temperature			
				1.0	24.0	Fast	Half mix	Room Temperature			
				0.5	25.0	Fast	Half mix	Room Temperature			
			Collect Beads 3x	0.5	26.5	Slow	Full volume	Room Temperature			
4	Wash 2	Wash 3	Release beads	0.5	27.0	Fast	Bottom mix	Room Temperature	80% Ethanol	1000	1000
			Wash Loop 3x	0.5	27.5	Fast	Bottom mix	Room Temperature			
				1.0	28.5	Fast	Half mix	Room Temperature			
				0.5	29.0	Fast	Full volume	Room Temperature			
			Collect Beads 3x	0.5	30.5	Slow	Full Volume	Room Temperature			
5	NA	Dry	Dry Step	6.0	36.5	Stop	Air dry above well	Room Temperature	N/A	N/A	N/A
6	Elution	Elute	Release beads	0.5	37.0	Fast	Bottom mix	72°C	MBL5	200	200
			Mix	5.0	42.0	Slow	Full volume	72°C			
				5.0	47.8	Fast	Full volume	72°C			
			Collect Beads 3x	0.5	47.5	Slow	3 passes	Room Temperature			

DNA Yield and Purity

The quantity and purity of the eluted DNA were evaluated by measuring the A_{260}/A_{280} absorbance ratio using a Take3 microplate reader (Biotek).

RESULTS

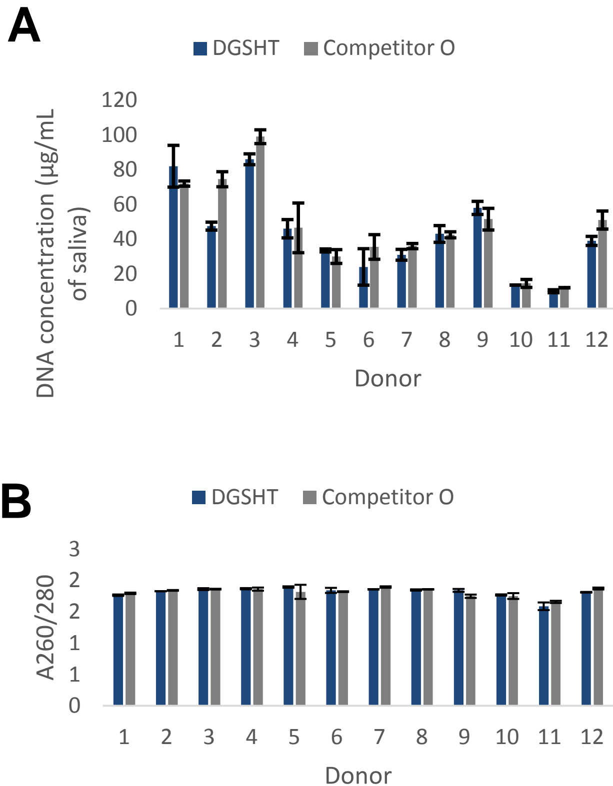

Figure 1: Yield of dsDNA isolated from saliva using DNAgard® Saliva HT (DGSHT) or Competitor O and manual NucleoMag® purification. *Saliva samples from 12 donors were mixed with stabilizers and the DNA purified. DNA concentration (A) and purity (B) were measured using UV absorbance.*

SUMMARY

Samples purified after storage in DNAgard® Saliva *HT* using the NucleoMag® extraction technology exhibit yield and purity comparable to samples stored in Competitor O's stabilizer (Figure 1). Use of the protocols provided should allow users to rapidly purify DNA from saliva using the KingFisher™ liquid handling system or similar platforms. Combined with the use of upstream liquid handling systems to remove samples directly from the DNAgard® Saliva *HT* tubes, users now have the tools needed to rapidly process saliva samples in high throughput systems without the need for extra decapping steps or the errors encountered with 1D barcode readers.

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