BioSPE™ PureGenNucleo cfDNA for cell-free DNA/RNA isolation from urine samples

This application note describes a ready-to-use kit for the efficient isolation, purification, and concentration of circulating cell-free DNA and RNA from human urine samples. The method relies on a sample preparation with different buffers prior to DNA/RNA isolation on BioSPE™ PureGenNucleo cfDNA spin columns.

Cervical cancer is a leading cause of gynecological cancer death in the world. Human papillomavirus (HPV) infection is a major causal factor. All cervical cancers are positive for one of the high-risk HPV subtypes. Patients diagnosed with high-grade CIN need to be monitored throughout their lives to detect possible relapse or development of invasive cancer. Various blood-based liquid biopsy approaches have shown great promise as an easily accessible, minimally invasive tool for detection of precancerous lesions. HPV status can be analyzed in patient's urine and may serve as a monitoring tool.

The extraction of DNA from biological matrices is usually performed using an extraction column in the presence of a chaotropic agent. A complete kit with all the required reagents (new buffer solutions) has been developed. To mimic patient samples, healthy control urine was spiked with fragmented DNA extracted from Hela cells harboring HPV-18 sequences.

Proceeding of the experiment and recoveries

Sample preparation:

1. In a centrifuge tube, add **1mL** of urine spiked with **10µL** of DNA at 23.2ng/µL (130bp in average).
2. Add **125µL** of Proteinase K to the tube.
3. Add **1mL** of lysis buffer containing **1µg** of carrier RNA.
4. Vortex for 30 seconds.
5. Incubate at 60°C for 30 minutes.
6. Add **3.6mL** of binding buffer to the tube and vortex for 15-30 seconds.
7. Incubate the mixture into the tube for 5 minutes on ice.

Purification with BioSPE™ PureGenNucleo cfDNA spin columns

Loading

1. The mixture of urine and buffers

Washing

1. **600µL** of wash buffer 1
2. **750µL** of wash buffer 2
3. **750µL** of ethanol (96-100%)

8. Remove the BioSPE™ PureGenNucleo cfDNA spin column from vacuum manifold and put it into a 2mL centrifuge tube.
9. Centrifuge the spin column at 20 000 x g for 3min.
10. Place the spin column into a new 2mL centrifuge tube and incubate 10 minutes at 56°C.
11. Place the spin column into a new 2mL centrifuge tube and add **60µL** of elution buffer.
12. Incubate at room temperature for 3 minutes.
13. Centrifuge 20 000 x g for 1 minute.
14. Repeat steps 11 to 13 a second time.

Conditions of analysis

Elutions are firstly analysed with a Qubit 4 to measure the DNA concentration, and then in Tape Station and ddPCR to obtain more information about the size of DNA fragments and their nature.

In the case of ddPCR analysis, HPV-16 and HPV-18 probes and primers were used. A volume of elution was taken to obtain 20ng of DNA for ddPCR analysis. The method is described below.

PCR program:

- 95°C for 10min
- 40 cycles: 94°C for 30 seconds and then 60°C for 1 minutes
- 98°C for 10min
- 10°C for an infinite time

Results

The samples were simultaneously analysed by Qubit 4. The results obtained are presented in the table below.

DNA (urine spiked at 0,23µg/mL)	1mL of urine		
	[C] in blank (ng/µL)	%Recovery	%RSD (n=3)
Doped	0,492	81%	5%

Table 1. Recovery obtained for 1mL of extracted urine.

A second analysis consisted of electrophoresis on an Agilent TapeStation system to determine the size of the DNA fragments retained, knowing that the samples were spiked with DNA with an average of 130bp. The results are presented below.

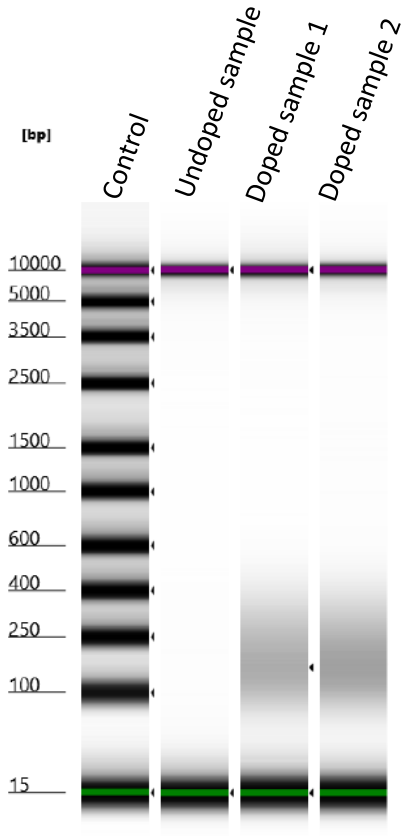

Figure 1. Results in Tape Station of undoped and doped urine.

A retention of DNA fragments of around 150bp is observed, corresponding to HPV DNA spiked during urine sample preparation.

To be sure of the presence of HPV DNA, a ddPCR was realized on the elution fraction.

DNA (urine spiked at 0,23µg/mL)	1mL of urine		
	[C] in blank (copy/µL)	Copy of HPV DNA/µL	%RSD (n=2)
Doped	0	444,5	1%

Table 2. Number of HPV DNA copy/µL obtained for 1mL of extracted urine.

This analysis confirmed the presence of HPV DNA with an average of 444,5 copy/µL.

CONCLUSION

BioSPE™ PureGenNucleo cfDNA for cell-free DNA/RNA isolation has been successfully used for the purification and extraction of DNA in urine samples. The method showed excellent performances with recovery rates averaging **81%**.

Furthermore, the use of Tape Station confirmed the presence of DNA at 130bp and ddPCR analysis confirmed the presence of HPV DNA with an average of 444,5copy/µL.

Part number of products used in this application note:		
Product:	**Preps:**	**Part number:**
BioSPE™ PureGenNucleo cfDNA mini spin columns	10	Spin-PureGenNucleo-cfDNA.S.10
BioSPE™ PureGenNucleo cfDNA mini spin columns	50	Spin-PureGenNucleo-cfDNA.S.50