BioSPE™ PureGenNucleo cfDNA for cell-free DNA/RNA isolation from plasma 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 plasma 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 plasma 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 plasma 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 plasma spiked with **10µL** of DNA at 23.2ng/µL (130bp in average).
2. Add **100µL** of Proteinase K to the tube.
3. Add **800µL** of lysis buffer containing **1µg** of carrier RNA.
4. Vortex for 30 seconds.
5. Incubate at 60°C for 30 minutes.
6. Add **1.8mL** 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 column

Loading

1. The mixture of plasma 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 for 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 at 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

Comparison of the AFFINISEP kit and a competitor's kit:

To assess the efficiency and selectivity of the AFFINISEP kit, it was compared to a competitor's kit, each following its own complete protocol (buffers and spins). For this comparison experiment, 232ng of HPV DNA (130bp on average) were added to plasma samples and were extracted from 1mL or 5mL of plasma (the buffer volumes used are proportional to the protocol for 1mL) with each kit.

Elutions from each protocol were analyzed with a Qubit 4, and recovery rates were calculated and presented below.

DNA (plasma spiked at 0,23µg/mL)	1mL of plasma		
	[C] in blank (ng/µL)	%Recovery	%RSD (n=3)
AFFINISEP	1,504	76%	4%
Competitor	1,797	75%	0%

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

DNA (plasma spiked at 0,23µg/mL)	5mL of plasma	
	%Recovery	%RSD (n=3)
AFFINISEP	104%	1%
Competitor	168%	2%

Table 2. Recovery obtained for 5mL of extracted plasma.

The AFFINISEP kit has a DNA extraction yield of almost 100%, while recovery was above 165% for the competitor's kit. These values can be explained by the initial presence of DNA in the plasma, and further analyses have been carried out to obtain more information on the nature of the DNA retained.

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.

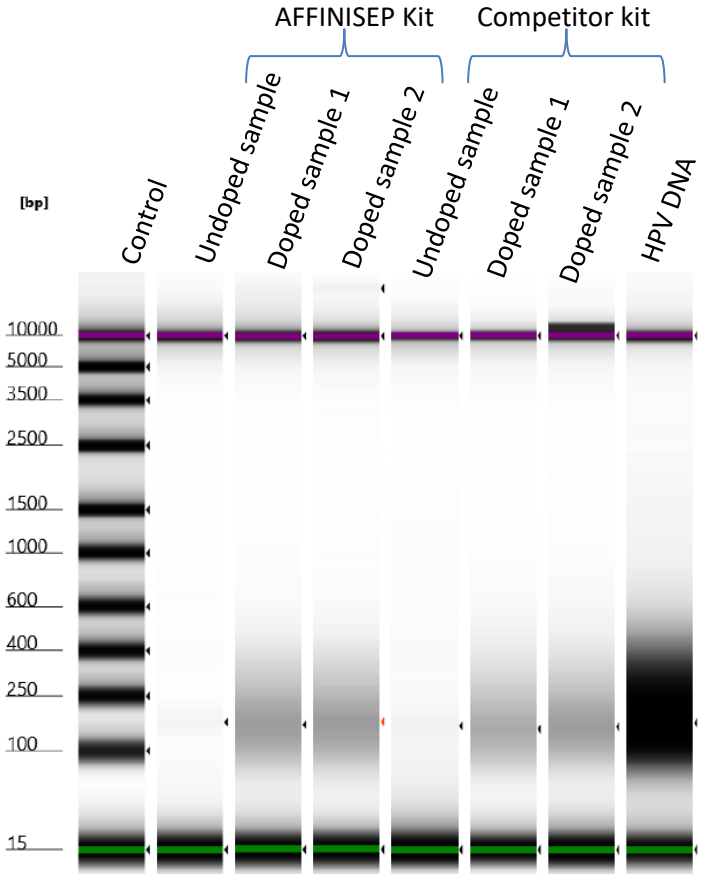

Figure 1. Comparison in Tape Station of AFFINISEP kit and a competitor for 1mL of plasma.

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

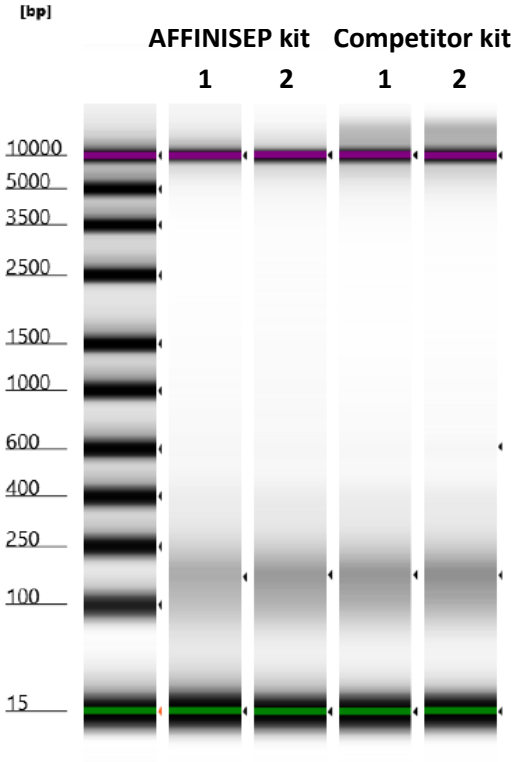

Figure 2. Comparison in Tape Station of AFFINISEP kit and a competitor for 5mL of plasma.

In both cases with 5mL of plasma, a retention of DNA fragments of around 100bp is observed, corresponding to HPV DNA spiked during plasma sample preparation. However, in the case of the competitor kit, much larger DNA fragments are observed at around 10 000bp, corresponding to genomic DNA fragments initially present in the plasma. In the AFFINISEP kit, this retention is not observed, thus demonstrating better selectivity for short fragments (around a hundred base pairs). These results explain the yields superior to 100% measured for the competing kit using Qubit.

To ensure that the amount of HPV DNA was efficiently retained, a ddPCR was carried out to determine the retention proportion with both kits for 1mL of spiked plasma.

DNA (plasma spiked at 0,23µg/mL)	1mL of plasma		
	[C] in blank (copy/µL)	Copy of HPV DNA/µL	%RSD (n=2)
AFFINISEP	0	446,5	9%
A competitor	0	444,5	1%

Table 3. Number of HPV DNA copy/µL obtained for 1mL of extracted plasma.

This analysis shows similar HPV DNA retention with both kits.

CONCLUSION

BioSPE™ PureGenNucleo cfDNA for cell-free DNA/RNA isolation has been successfully used for the purification and extraction of DNA in plasma samples. The method showed excellent performances from 1mL to 5mL of plasma with recovery rates averaging **76% to 104%**.

Furthermore, the use of Tape Station confirmed the presence of DNA at 130bp and showed a better selectivity for the short fragments than the competitor kit. The ddPCR analysis confirmed the presence of HPV DNA with an average of 446,5copy/µL like competitor kit.

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