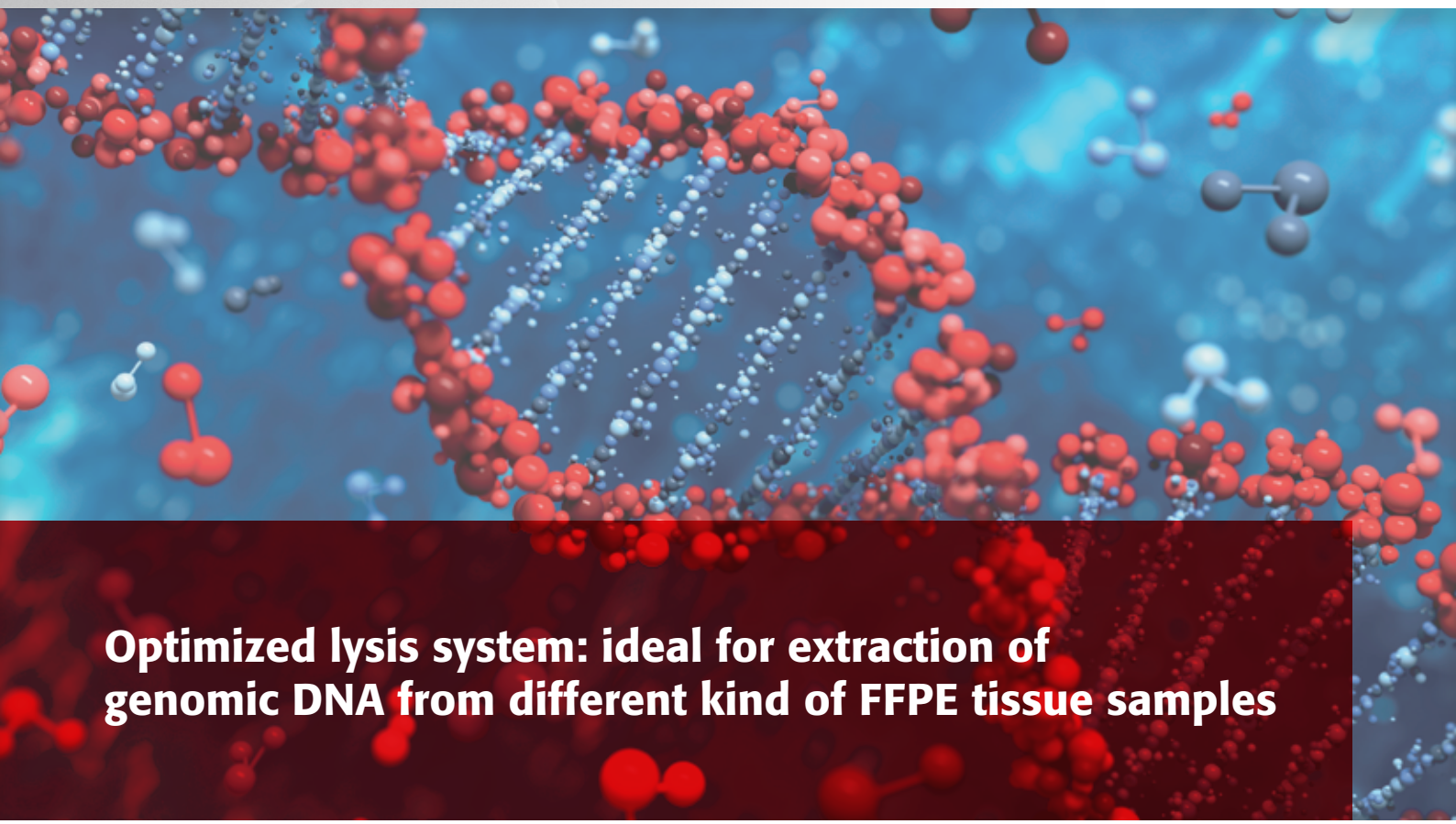
APPLICATION NOTE
blackPREP FFPE DNA Kit

analytikjena



Optimized lysis system: ideal for extraction of genomic DNA from different kind of FFPE tissue samples

1. INTRODUCTION

Analytik Jena's blackPREP FFPE DNA Kit is optimized for DNA extraction from a variety of FFPE (formalin-fixed, paraffin-embedded) tissue sample, e.g. adipose tissue, pancreatic tissue or placenta as starting materials.

FFPE tissue samples are an important source of DNA for retrospective studies of gene expression patterns or mutation analysis. Nevertheless the amount of the tissue and paraffin varies from sample to sample and the DNA is modified due to the fixation by formaldehyde. Based on patented low salt DC-Technology® and the usage of reliable Spin Filter columns the blackPREP FFPE DNA Kit is the optimal tool to extract high quality DNA from different kind of FFPE tissue with varying paraffin amount.

2. MATERIAL AND METHODS

- blackPREP FFPE DNA Kit
- FFPE tissue samples
- Thermal mixer (e.g. BioShake iQ)
- Centrifuge
- Spectrophotometer (e.g. ScanDrop®)
- Real-time thermal cycler (e.g. qTOWER 2 or TOptical)
- Equipment for gel electrophoresis analysis

Lysis

Different amounts of FFPE samples (placenta tissue, table 1) were lysed by using 400 µl Lysis Buffer MA included in the Kit. Proteinase K (40 µl) was added in order to digest the protein from the sample. Lysis was carried out for 1 h at 65 °C using BioShake iQ thermal mixer followed by an incubation at 90 °C to decrosslink.

Sample ID	No. of sections	Thickness	Weight of tissue	Paraffin surface
A	4	10 µm	~2 mg	1.200 mm^2
B	2	10 µm	~2 mg	600 mm^2
C	1	10 µm	~2 mg	300 mm^2
D	2	10 µm	~2 mg	600 mm^2
E	3	10 µm	~2 mg	900 mm^2

Table 1: Sample specification

Extraction

After lysis and decrosslink steps, the samples were cooled down at room temperature for 5 min and centrifuged at maximum speed for 2 min. The supernatant was transferred in a new reaction tube. 400 µl of ethanol absolute was added and mixed to adjust binding conditions. Subsequently the whole sample was applied to the Spin Filter (black). After binding of DNA to Spin Filter membrane the DNA was washed and centrifuged at maximum speed for 3 min to remove all traces of ethanol. Finally, the nucleic acids were eluted in 100 µl Elution Buffer.

3. RESULTS AND DISCUSSION

Following to the extraction process, the amount of isolated DNA (eluted in 100 µl Elution Buffer) was measured by spectrophotometric method and subsequent amplification of a human specific target sequence by Real-Time PCR using qTOWER 2. The extracted DNA was also analyzed by Gel electrophoresis. Table 2 shows purity (A260:280 and A260:230) and concentration determined by using ScanDrop® spectrophotometer.

Sample ID	Ø Concentration	A_{260}:A_{280}	A_{260}:A_{260}	Ct values	
A	88.24 ng/µl	1.74	2.13	26.60	26.49
B	91.2 ng/µl	1.82	2.11	26.51	26.78
C	88.42 ng/l	1.77	1.90	26.50	26.84
D	191.76 ng/µl	1.79	2.13	26.62	25.98
E	261.14 ng/µl	1.79	2.11	25.46	25.76

Table 2: Summary of results

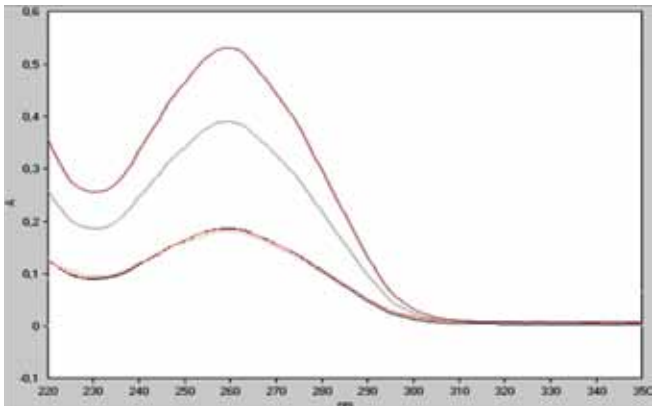


Fig. 1: UVVIS spectra from 220 to 350 nm for each sample (light red : Sample A, black : sample B, light grey : sample C, dark grey : sample D and dark red: sample E)

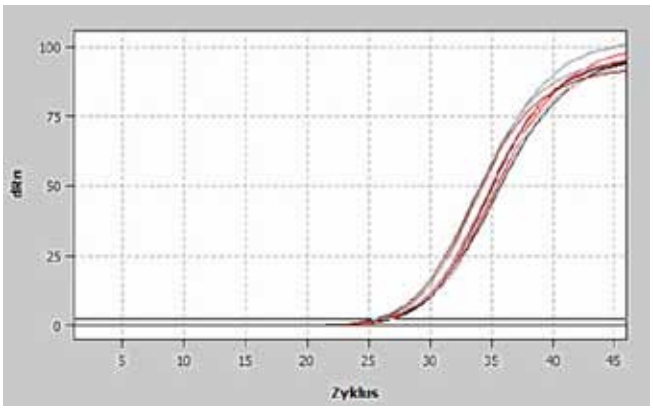


Fig. 2: Real-time amplification plots of a human specific target sequence. (light red : Sample A, black : sample B, light grey : sample C, dark grey : sample D and dark red : sample E)

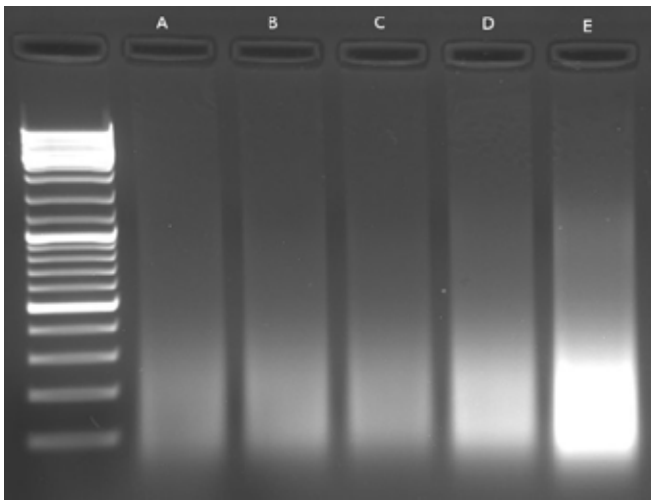


Fig. 3: 10 µl of extracted DNA were applied on a 2 % agarose gel

The Ct values of the double determination of each FFPE tissue sample correspond ideally to the yield measured by UVVIS spectrophotometer and to the bands shown in the agarose gel.

4. CONCLUSION

High purity of extracted DNA from FFPE tissue samples is essential for further analysis. FFPE tissue samples strongly varies in amount of tissue and paraffin. Specially optimized lysis conditions, as realized in the blackPREP FFPE DNA Kit, allow a very effective lysis of the tissue and an efficient deparaffinization even in case of high tissue and paraffin content. The extracted genomic DNA is free of paraffin and other possible inhibitors to ensure reliable results of further downstream applications like PCR, real-time PCR or SNP analysis.

Reference: AN_0113_0021_en_150721.docx

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