WHITE PAPER

Which FFPE Kit is for Me? An Investigation of Formalin-Fixed and Paraffin-Embedded (FFPE) DNA Extraction Kits





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1.0 Experimental Overview and Conclusions

Formalin-fixed and paraffin-embedded (FFPE) tissues are used routinely for research and the diagnosis of disease. Due to extensive protein-nucleic acid and protein-protein crosslinking in these tissues, it is a common challenge for bench scientists to extract high-quality template from FFPE samples that is usable for downstream manipulation. There are many commercially-available kits designed for this purpose that vary in price, but is there a difference between an inexpensive, quick kit and a pricier kit with a longer processing time? Azenta Life Sciences' R&D scientists wanted to determine if there were any differences between kits in ease of processing, template quality, and template yields. In this study, we tested 6 different FFPE kits from various manufacturers and extracted DNA from a 6-year old FFPE breast cancer sample to evaluate protocol ease and speed, DNA quality, and DNA yield (Table 1).

Key: Fair	Good	Excelle	ent			
FFPE Kit	A blackPREP FFPE DNA Kit/innuPREP (50 rxns)	M NucleoSpin® DNA FFPE XS (50 rxns)	O E.Z.N.A. [®] FFPE DNA kit (50 rxns)	P ReliaPrep™ FFPE gDNA Miniprep System (100 rxns)	Q QIAamp® DNA FFPE Tissue Kit (50 rxns)	R High Pure FFPET DNA Isolation Kit (50 rxns)
Supplier	Analytik Jena®	Macherey- Nagel®	OMEGA Bio-tek®	Promega®	Qiagen®	Roche®
Catalog #	845-BP- 0020050	740980.5	D3399-01	Z1002	56404	6650775001
Manufacturer- recommended lysis time (1-3 hr)						
Total time for isolation (2.5-5 hr)						
DNA yield, recommended lysis time						
DNA yield overnight						
DNA integrity number (DIN)						
Quality as PCR template						
Additional deparaffinization requirements?						
List price (USD) per reaction						

Table 1. FFPE kit testing results – at a glance.

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As expected, there was a wide range of results generated from the different kits tested. For DNA yield, our results indicate that the more expensive, lengthier extraction kits provide measurably higher extraction efficiencies. The Macherey-Nagel kit failed in our experiments to provide enough template for further testing, and was not included in the PCR experiment due to this failure. The Analytik Jena kit had stand-out DNA integrity number results compared to other kits, as well as having the bonus of no additional deparaffinization step required. All kits besides the Macherey-Nagel performed at least moderately well in the DNA integrity test and in generating DNA with sufficient quality to use as a template in a PCR reaction.

In conclusion, if you need the highest amount of DNA yield possible, the extra cost, time, and deparaffinization step required by the "gold standard", name-brand kits may be worth it. However, if total yield is not as important, but protocol ease, processing time, and high-quality template is of higher value in your experiments, the Analytik Jena kit seems to be a viable alternative to investigate further. It is also worth noting that these data only represent results from one source of template (6-year-old breast cancer tissue); other tissues and samples may perform differently.

2.0 Materials and Methods

The same sample was used for all testing: FFPE breast tissue from a 42-year-old female diagnosed with breast carcinoma. The sample was processed in May 2010. At the time of testing, the sample was approximately 6.5 years old. The tissue section was 10 µm thick.

DNA extractions using each kit were performed according to manufacturers' protocols. The protocols all recommended either a 1-hour or a 3-hour lysis, which was tested as the "standard" protocol. Additionally, each kit was used with an extended lysis treatment, in which the lysis reaction was allowed to occur overnight. All kits were tested with the recommended paraffin dissolving agent (either manufacturer-supplied, xylene, heat, or mineral oil), with the exception of the Analytik Jena kit. The Analytik kit needed no deparaffinization step. These extractions were used as templates for the following analysis parameters.

2.1 DNA Yield

DNA yields were measured for all treatments across each kit. DNA concentration was determined using the Qubit[®] dsDNA assay kit. To obtain DNA yield, the measured DNA concentration was multiplied by the total elution volume.

2.2 DNA Integrity Number (DIN)

DNA Integrity Numbers (DIN) were measured for all treatments across each kit. DIN was measured using the Agilent[®] 2200 TapeStation[®] system with genomic DNA ScreenTape.

2.3 DNA Quality as PCR Reaction Template

DNA quality was measured for all treatments across all kits except the Macherey-Nagel kit. The MN kit did not produce a sufficient amount of template for a viable PCR reaction. 15 ng of each treatment from each kit was used as starting template in a standard PCR reaction. The PCR reactions were set up to amplify a 402 bp sequence of the human PTEN gene using the following primers: PTEN_Forward (5' ATTTCCATCCTGCAGAAGAAG 3') and PTEN_(5' TATAGATAGCCTAAGAAAGCAATCG 3'). PCR cycling conditions followed the enzyme manufacturer's guidelines and were standardized to a 25-cycle reaction in a 20 µl reaction volume. PCR reactions were visualized on a 1% agarose gel and band intensities were compared visually. The PCR reactions were also purified enzymatically and analyzed via Sanger sequencing. All sequences tested exhibited the expected PTEN gene sequence.

3.0 Results

3.1 DNA Yield

The amount of DNA extracted from human breast cancer FFPE tissue using six different DNA extraction kits is shown in Figure 1. Both standard protocol (1 hour or 3 hour) and extended lysis protocol (overnight) were tested.

The data show that all tested kits produced higher DNA yields for overnight digestions compared to 1-hour and 3-hour digestions. For overnight digestion, QIAamp DNA FFPE Tissue Kit from Qiagen produced the highest DNA yield. For the 1-hour digestion, the blackPREP FFPE DNA Kit/innuPREP from Analytik Jena produced the highest yield. The Macherey-Nagel kit noticeably produced minimal yield (<50 ng) in this experiment, regardless of lysis time.



Figure 1. DNA yield by extraction kit and lysis treatment. Key: A = Analytik Jena; M = Macherey-Nagel; O = OMEGA Bio-tek; P = Promega; Q = Qiagen; and R = Roche.

3.2 DNA Integrity Number (DIN)

The integrity of the DNA obtained from an extraction kit affects the success of downstream applications. The DNA integrity number (DIN) ranges from 1 to 10 with 1 being the worst and 10 being the best. The DINs of the gDNA from each extraction kit treatment are presented in Figure 2. We tested both the standard (1 hour or 3 hour) and extended lysis protocols (overnight). As expected from an archived tissue of more than 5 years old, the DIN of less than 3 attained by all the kits suggests that the DNA sample had degraded gradually over time.



Figure 2. DNA integrity numbers of tested FFPE extraction kits. Key: A = Analytik Jena; M = Macherey-Nagel; O = OMEGA Bio-tek; P = Promega; Q = Qiagen; and R = Roche.

3.3 PCR Amplification of a PTEN Fragment

We amplified a fragment of the human phosphatase and tensin homolog (PTEN) gene using the gDNA from each extraction kit treatment as template. The Macherey-Nagel kit was not included due to low DNA yield. Extracts from both the standard protocol (1 hour or 3 hour) and the extended lysis protocol (overnight) were tested (Figure 3). The PCR products were purified and all sequenced to confirm the PTEN gene sequence. The quality of the gDNA extracted with the 5 kits tested was good enough to generate the target band in the PCR. However, the band intensity, which is an indicator of the integral copy number of the DNA template, differed from kit to kit. Overnight digestions with ReliaPrep FFPE gDNA Miniprep System (Promega), blackPREP FFPE DNA Kit/innuPREP (Analytik Jena), and High Pure FFPET DNA Isolation Kit (Roche) produced the strongest amplification results.

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Figure 3. Use of extracted DNA as PCR reaction template. Key: A = Analytik Jena; O = OMEGA Bio-tek; P = Promega; Q = Qiagen; and R = Roche.

FFPE Kit	Supplier	Cat. #	List Price (\$)	Price Per Reaction (\$)
blackPREP FFPE DNA Kit/innuPREP (50)	Analytik Jena	845-BP-0020050	176	3.52
NucleoSpin (R) DNA FFPE XS (50)	Macherey-Nagel	740981	193	3.86
E.Z.N.A. FFPE DNA kit (50)	OMEGA Bio-tek	D3399-01	134	2.68
ReliaPrep FFPE gDNA Miniprep System (100)	Promega	A2352	414	4.14
QIAamp DNA FFPE Tissue Kit (50)	Qiagen	56404	233	4.66
High Pure FFPET DNA Isolation Kit (50)	Roche	6650767001	292	5.84

Table 2. FFPE kits and their suppliers.

5.0 Appendix

Key: Fair	Good	Excelle	ent			
FFPE Kit	A blackPREP FFPE DNA Kit/innuPREP (50 rxns	M NucleoSpin DNA FFPE XS (50 rxns)	O E.Z.N.A. FFPE DNA kit (50 rxns)	P ReliaPrep FFPE gDNA Miniprep System (100 rxns)	Q QIAamp DNA FFPE Tissue Kit (50 rxns)	R High Pure FFPET DNA Isolation Kit (50 rxns)
Supplier	Analytik Jena	Macherey- Nagel	OMEGA Bio-tek	Promega	Qiagen	Roche
Catalog #	845-BP- 0020050	740980.5	D3399-01	Z1002	56404	6650775001
Manufacturer- recommended lysis time (1-3 hr)	1	3	3	1	1	1
Total time for isolation (2.5-5 hr)	2.5	4.5	5	2.5	2.5	3
DNA yield, recommended lysis time	225 ng	25 ng	120 ng	200 ng	175 ng	140 ng
DNA yield overnight	275 ng	25 ng	275 ng	390 ng	425 ng	310 ng
DNA integrity number (DIN)	2	1	1.5	1.5	1.5	1.5
Quality as PCR template						
Additional deparaffinization requirements?	No	Yes	Yes	Yes	Yes	Yes
List price (USD) per reaction	\$3.52	\$3.86	\$2.68	\$4.14	\$4.66	\$5.84

Table 3. FFPE kit testing results - in detail.

Disclaimer: In this product review, we mention and discuss several commercially available FFPE extraction kits and their corresponding suppliers. The inclusion of these proprietary DNA kits and vendors is not intended to reflect their importance, nor is it intended as an endorsement by Azenta Life Sciences. The DNA kits and suppliers mentioned are provided for informational and noncommercial use only. Any reference to any vendor, process, service, or enzyme by trade name, trademark, or manufacturer or otherwise does not constitute or imply the endorsement, recommendation, favoring, or approval of Azenta Life Sciences.



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