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Commercially available kits for manual and automatic extraction of nucleic acids from formalin-fixed, paraffin-embedded (FFPE) tissues

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Abstract

Introduction: Formalin-fixed, paraffin-embedded (FFPE) tissues represent an invaluable source for diagnostic purposes when fresh clinical material is unavailable, and also for molecular and epidemiological studies. The recovery of nucleic acids from FFPE tissues is particularly challenging, and several in-house methods have been developed for this purpose over the last three decades. Recently, several commercial kits specifically developed for DNA and/or RNA extraction from FFPE tissues have been introduced to the market, but their inventory is not available in peer-reviewed literature.
Methods: This article provides the first comprehensive inventory of commercial FFPE DNA/RNA extraction kits currently available on the market and describes their basic characteristics and features.
Results: A total of 69 commercial kits from 43 companies were identified. Thirty-five kits were developed specifically for DNA extraction, 22 for RNA extraction, and 12 for both DNA and RNA extraction. Only two commercial kits allow full automation of the entire nucleic acid extraction procedure. The tissue deparaffinization step is omitted in many protocols by melting paraffin directly in a tissue lysis buffer. Purification of the released nucleic acids is mainly based on silica or resin adsorption technology. A formalin reverse cross-linking step to increase the quality of extracted DNA and RNA is an intrinsic part of over half of the kits identified.
Conclusions: It is hope that this comprehensive list of available commercial kits for extracting nucleic acids from FFPE will encourage researchers to strongly consider using them in diagnostic and research settings instead of old-fashioned, crude, and probably less effective in-house methods.

Keywords: archival tissues specimens, formalin-fixed, paraffin-embedded tissue, FFPE, nucleic acid extraction, DNA, RNA

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Introduction

Formalin-fixed, paraffin-embedded (FFPE) tissues stored in pathology departments worldwide represent an invaluable source for diagnostic purposes when fresh clinical material is unavailable and also for molecular and epidemiological studies. However, working with nucleic acids extracted from FFPE tissue specimens is particularly challenging due to cross-linking of bio-molecules and fragmentation of nucleic acids. Several factors affect the quality of nucleic acids obtained from FFPE tissues, most notably the pH of the fixative, the duration of tissue fixation, the age and storage conditions of FFPE tissue blocks, and the method used for their extraction (1). The integrity of DNA/RNA is generally affected by a multitude of these factors, generating a large diversity of sample quality and highly variable target amplification (2).

Finding a suitable method for extracting nucleic acids from a particular clinical specimen is a prerequisite for successful subsequent testing with molecular methods such as those based on polymerase chain reaction (PCR). During the last three decades, many specific approaches for extracting DNA/RNA from FFPE tissues, which is then used for PCR, have been reported. In the early 1990s, several protocols were developed for rapid extraction of DNA and/or RNA from FFPE specimens, including boiling FFPE tissue sections in chelating resin solution or distilled water (3, 4), incubation in sodium dodecyl sulfate (SDS) or alkali buffers combined with phenol/chloroform purification (5, 6), and sonication (7), all with varying degrees of success. Proteolytic treatment with proteinase K with or without subsequent organic solvent purification has been one of the most frequently used methods for DNA/RNA extraction from FFPE specimens, generally resulting in a satisfactory DNA/RNA yield and integrity for subsequent molecular analyses (1). Introduction of silica adsorption technology in 1996 (8) has greatly revolutionized purification of nucleic acids; for example, by improving the purity of DNA/RNA molecules, reducing preparation times, eliminating the need for toxic chemicals, and making it possible to automate the entire procedure. Since then, several silica adsorption–based commercial kits have been developed for extracting DNA and/or RNA molecules from various fresh clinical specimens, including tissue, mucosal/skin swabs, blood, liquor, and various body fluids. Moreover, these particular kits (not originally developed for FFPE tissues) have also been frequently used for nucleic acid extraction from FFPE tissue specimens, some employing innovative modifications of the original extraction procedure, such as pretreatment of paraffin sections with elevated temperatures (9), melting of paraffin directly in tissue lysis buffers (10), and/or addition of a reverse formalin cross-linking step (10).

Several commercial kits specifically designed for nucleic acid extraction from FFPE tissue specimens have been recently introduced to the market and are gradually being used in research on FFPE (11, 12). To the best of our knowledge, an inventory of commercial kits specifically designed for nucleic acid extraction from FFPE is currently not available in peer-reviewed literature. Thus, this review provides the first comprehensive inventory of commercial manual and automatic FFPE DNA/RNA extraction kits and systems currently available on the market and describes their basic characteristics and features.

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Methods

The data for this review were retrieved through a detailed search of Medline/Pubmed, Web of Science, Scopus, Google Scholar, Google, and Bing between July 1 and July 30, 2015. In addition, official websites of companies manufacturing nucleic acid extraction kits were searched in detail. Despite our best efforts, due to rapid developments in FFPE nucleic acid extraction kits and a lack of corresponding peer-reviewed publications, it is likely that not all kits currently available on the global market were identified and the omission of any particular available kit is unintentional.

Results

FFPE nucleic acid extraction kits

As summarized in Table 1, we identified a total of 69 commercial kits specifically designed for nucleic acid extraction from FFPE tissue specimens from 43 companies that are currently available on the market. Of these, 35 kits were specifically developed for DNA extraction, 22 for RNA extraction, and 12 for both DNA and RNA extraction (Table 1). Some kits allow the recovery of RNA throughout a range of sizes, including smaller microRNAs (miRNAs) and small interfering RNAs (siRNAs). Fifty-one kits were designed for manual, mostly column-based DNA/RNA extraction, eleven for manual or automated extraction, five for automated extraction, and two for fully automated DNA/RNA extraction. Interestingly, the majority of kits identified were launched in the last few years, and with a few exceptions (e.g., the Qiagen QIAamp DNA FFPE Tissue Kit) they consequently lack documented performance evaluation in peer-reviewed literature.

In the majority of kits identified, the digestion of standardized amounts of FFPE tissue, measured in tissue sections of various thickness or milligrams, is performed in a tissue lysis buffer containing proteinase K (Table 1). Exceptions to these include the RealLine FFPET DNA Extraction Kit (Bioron Diagnostics, Ludwigshafen, Germany), Geno-Prep FFPE DNA Kit (Genolution Pharmaceuticals, Seoul, Korea), and TaKaRa DEXPAT Easy (TaKaRa, Shiga, Japan), for which tissue lysis is performed without enzyme digestion. Deparaffinization of FFPE tissue sections using xylene is still one of the most frequent recommendations. However, to eliminate the use of flammable and malodorous xylene or d-limonene (Hemo-De), some companies have developed special, presumably less toxic, chemicals, making possible fast and efficient solubilization, phase separation, and removal of paraffin, such as Q-solution (TrimGen, Sparks, MD, USA), Deparaffinization solution (Qiagen, Hilden, Germany), BiOstic Paraffin Removal Reagent (MO BIO Laboratories, Carlsbad, CA, USA), and Paraffin Dissolver A (Exiqon, Vedbaek, Denmark). Because deparaffinization is laborious and can result in severe tissue loss and consequently lower DNA/RNA yield (9, 10), this step was omitted in many protocols, allowing melting of paraffin directly in tissue lysis buffers. However, the usual recommendation in this case is to trim away excess paraffin during tissue sectioning prior to starting tissue lysis. An incubation step at elevated temperatures (e.g., 70–90 °C for various times) following tissue lysis to partially remove formalin cross-links of the released DNA/RNA, thus improving the quality and DNA/RNA performance in downstream assays (1, 13), was identified in 41 kits with available information.

Of the available manual kits, the recently launched FFPE DNA Extraction Kit (Roche Molecular Systems Inc., Alameda, CA, USA) allows extraction of DNA from FFPE tissues in two steps in only 67 minutes using inventory heat elution technology. FFPE tissue sections including paraffin are placed into a specially designed heat-elution column containing resin, which is first heated to 56 °C for 1 hour to lyse the tissue. Following tissue lysis, pressure is created in the column as the liquid is briefly incubated at 98 °C, allowing the elution and purification of genomic material.

Automation of extraction of nucleic acids from FFPE

In comparison to the manual procedure, automated protocols may produce better nucleic acid extraction reproducibility, require less tissue input, and/or require less hands-on time (14, 15). As already mentioned, we identified 16 FFPE DNA/RNA kits that were developed to work with systems that allow automated extraction of nucleic acids (Table 1). In most of the cases, tissue digestion with proteinase K is performed in an external water bath or a rocking platform until the sample is completely lysed. The tissue digest without tissue debris is then manually transferred to a fully automated instrument containing ready-to-use reagents or cartridges with buffers optimized for one-step extraction of DNA and/or RNA, usually with the use of magnetized beads. Interestingly, two of the nucleic acid extraction systems identified have an integrated (combined) paraffin-melting and tissue-lysis step, thus allowing full automation of the entire nucleic acid extraction procedure (Table 1).

The first, the Siemens system (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), employs an automated Tissue Preparation System (Hamilton MICROLAB STARlet IVD instrument) and the VERSANT Tissue Preparation Reagents kit with universal chemistry for simultaneous co-isolation of DNA and RNA from a single FFPE tissue section in a single step. This extraction system is based on iron oxide beads coated with a nanolayer of silica that are homogenous in shape and size (spherical, < 1 µm), which allows improved reproducibility, recovery, and quality of nucleic acids (16, 17). In the first step, simultaneous paraffin melting and FFPE tissue lysis are performed, followed by non-specific binding of tissue debris to silica beads under non-chaotropic conditions. Removal of the remaining undigested tissue is necessary to achieve effective and complete automation because it may interfere with accurate liquid handling and result in clogging pipette tips (17). A xylene-free deparaffinization step, based on hydrophobic absorption of molten paraffin into the inner polypropylene wall of the sample tube during the lysis process, further allows automation of the entire procedure (17). In the following step, the lysis fluid containing DNA/RNA is transferred to a chaotropic buffer containing fresh silica beads. Following binding and washing, pure DNA/RNA is eluted from silica beads and stored until downstream applications. The system is able to process a total of 48 FFPE samples (one or more 5–10 µm thick FFPE tissue sections) in less than 4 hours, including a 30-minute incubation step for DNase I digestion if pure RNA is required (17).

The second system, the MagCore system (MagCore, Châtel-St-Denis, Switzerland), employs an automated MagCore HF16 Automated DNA/RNA Purification System and Genomic DNA FFPE One-Step Kit and makes possible single-step extraction of total DNA from one to five FFPE tissue sections. In the first step, simultaneous paraffin melting and FFPE tissue lysis is performed, which is followed by DNA purification using cellulose-coated magnetic beads; this particular technology is characterized by high binding capacity and high purity of the nucleic acids obtained. The MagCore system is able to process up to 16 FFPE samples (up to 5 µm thick FFPE tissue sections) in less than 70 minutes.

Table 1 | List of commercially available kits for extracting DNA and/or RNA from FFPE tissue specimens. *(continued on next page)*

No.	Kit	Manufacturer	DNA/RNA extraction manipulation	Instrument	Type of purification	Nucleic acids	Tissue amount	Deparaffinization	Proteinase K digestion	Reverse formalin cross-linking step
1	ArchivePure DNA Tissue Kit	5prime	manual	/	alcohol precipitation	DNA	5–10 mg	Hemo-De or xylene	Yes (55 °C)	No
2	AmoyDx FFPE DNA/ RNA Kit	AmoyDx	manual	/	columns, silica	DNA, RNA	2–5 sections (5–10 µm)	xylene	Yes (56 °C)	Yes
3	Absolutely RNA FFPE Kit	Agilent Technologies	manual	/	columns, silica	RNA	2 sections (≤ 10 µm)	D-limonene	Yes (55 °C)	No
4	ExpressArt FFPE Clear RNAready Plus Kit	Amsbio	manual	/	columns	RNA, miRNA	1–5 sections (≤ 10 µm)	FFPE Clear solution	Yes (55 °C)	Yes
5	ExpressArt FFPE Clear RNAready	Amsbio	manual	/	columns	RNA	1–5 sections (≤ 10 µm)	FFPE Clear solution	Yes (55 °C)	Yes
6	blackPREP FFPE DNA Kit	Analytikjena	manual	/	columns	DNA	2 sections (≤ 5 µm)	melting in tissue lysis buffer	Yes (50 °C)	Yes
7	AxyPrep Mag FFPE DNA-RNA	Axygen Biosciences	manual	/	magnetic beads	DNA, RNA	3–8 sections (5–10 µm)	xylene or melting in tissue lysis buffer	Yes (55 °C)	Yes
8	FFPE Tissue DNA Extraction Kit; Column; Magnetic Beads	BioChain	manual	/	columns/magnetic beads	DNA	1–5 sections (5–10 µm)	melting in tissue lysis buffer	Yes (56 °C)	Yes
9	EZgene FFPE DNA Kit	Biomiga	manual	/	columns	DNA	3–8 sections (10–20 um)	xylene	Yes (50 °C)	Yes
10	RealLine FFPET DNA Extraction Kit	BIORON Diagnostics	manual	/	alcohol precipitation	DNA	2 sections (≤ 10 µm)	melting in tissue lysis buffer	No, lysis in a NaOH solution/detergents	Yes
11	FFPE RNA/DNA Purification Plus Kit	Bio-Synthesis	manual	/	columns, resin	DNA, RNA, miRNA, siRNA	4 sections (< 20 µM)	xylene	Yes	Yes
12	FFPE DNA Purification Kit	Bio-Synthesis	manual	/	columns, resin	DNA	5 sections (< 20 µm)	xylene	Yes	Yes
13	Sampletype i-sep DL	Biotype	manual	/	columns	DNA	1–3 sections (≤ 15 mg)	BiOstic Paraffin Removal Reagent	Yes (56 °C)	No
14	GenSeq FFPE RNA Isolation Kit	CD genomics	manual	/	columns, silica	RNA	N/A	melting in tissue lysis buffer	Yes (60 °C)	Yes
15	truXTRA FFPE DNA Kit	Covaris	manual	/	columns	DNA	sections (15–25 µm or 2–5 mg)	melting in tissue lysis buffer following tissue processing with AFA technology	Yes (56 °C)	Yes
16	FFPE DNA Extraction kit	Diagenode	manual	/	columns	DNA	sections (≤ 10 µm)	melting in tissue lysis buffer following tissue sonication	Yes (56 °C)	Yes
17	QuickExtract FFPE DNA Extraction Kit	Epicentre	manual	/	no, crude extract	DNA	1–3 sections (5–10 µm)	melting in tissue lysis buffer	N/A (lysis performed at 56 °C)	Yes
18	QuickExtract FFPE RNA Extraction Kit	Epicentre	manual	/	no, crude extract	RNA	2–3 sections (5–10 µm)	melting in tissue lysis buffer	N/A (lysis performed at 56 °C)	Yes
19	miRCURY RNA Isolation Kit, FFPE	Exiqon	manual	/	columns, silica	RNA	5 sections (10 µm)	Paraffin Dissolver A	Yes (56 °C)	Yes
20	XIT Genomic DNA from FFPE Tissue	G biosciences	manual	/	alcohol precipitation	DNA	≤ 10 mg	xylene	Yes (55 °C)	No
21	Geno-Prep FFPE DNA Kit	Genolution Pharmaceuticals	manual	/	magnetic beads/ columns	DNA	3–4 sections (≤ 35 mg)	xylene	No, heat induced lysis	No

Table 1 | Continued.

No.	Kit	Manufacturer	DNA/RNA extraction manipulation	Instrument	Type of purification	Nucleic acids	Tissue amount	Deparaffinization	Proteinase K digestion	Reverse formalin cross-linking step
22	PureLink FFPE Total RNA Isolation Kit	Invitrogen	manual	/	columns, silica	RNA	3–8 sections (10 µm)	melting in tissue lysis buffer	Yes (60 °C)	No
23	Arcturus Paradise PLUS FFPE RNA Isolation Kit	Applied Biosystems	manual	/	columns	RNA	sections (≤ 7 µm)	xylene	Yes (37 °C)	No
24	RecoverAll Total Nucleic Acid Isolation Kit	Invitrogen	manual	/	columns, silica	DNA, RNA, miRNA	1–4 sections (20 µm)	xylene	Yes (50 °C)	No
25	FFPE DNA Extraction Kit	Roche, previously Lumora	manual	/	column, resin	DNA	N/A	melting in tissue lysis buffer	N/A (lysis performed at 56 °C)	No
26	NucleoSpin DNA FFPE XS	Macherey-Nagel	manual	/	columns, silica	DNA	sections (3–20 µm)	Paraffin Dissolver or xylene	Yes (room temperature)	Yes
27	NucleoSpin totalRNA FFPE	Macherey-Nagel	manual	/	columns, silica	RNA	sections (3–20 µm)	Paraffin Dissolver or xylene	Yes (56 °C)	Yes
28	BiOstic FFPE Tissue RNA Isolation Kit	MO BIO Laboratories	manual	/	columns, silica	RNA	1–5 sections (≤ 15 mg)	BiOstic Paraffin Removal Reagent or melting in tissue lysis buffer	Yes (60 °C)	Yes
29	BiOstic FFPE Tissue DNA Isolation Kit	MO BIO Laboratories	manual	/	columns, silica	DNA	1–5 sections (≤ 15 mg)	BiOstic Paraffin Removal Reagent or melting in tissue lysis buffer	Yes (55 °C)	Yes
30	FFPE RNA/DNA Purification Kit	Norgen Biotek	manual	/	columns, resin	DNA, RNA, siRNA, miRNA	5 sections (≤ 20 µm)	xylene	Yes (55 °C)	Yes
31	Prelude FFPE RNA Isolation Module	NuGEN	manual	/	columns, resin	RNA, siRNA, miRNA	5 sections (≤ 20 µm)	xylene	Yes (55 °C)	Yes
32	E.Z.N.A. FFPE DNA Kit	Omega bio-tek	manual	/	columns	DNA	3–8 sections (5–10 µm)	xylene	Yes (55 °C)	Yes
33	ReliaPrep FFPE gDNA Miniprep System	Promega	manual	/	columns	DNA	sections (5–50 µm)	mineral oil, xylene, or melting in tissue lysis buffer	Yes (56 °C)	Yes
34	ReliaPrep FFPE Total RNA Miniprep System	Promega	manual	/	columns	RNA	sections (5–50 µm)	mineral oil, xylene, or melting in tissue lysis buffer	Yes (56 °C)	Yes
35	High Pure FFPET RNA Isolation Kit	Roche	manual	/	columns, silica	RNA	sections (≤ 10 µm)	xylene	Yes (55 °C)	No
36	High Pure FFPE RNA Micro Kit	Roche	manual	/	columns, silica	RNA	sections (1–10 µm)	Hemo-De or xylene	Yes (55 °C)	No
37	High Pure FFPET DNA Isolation Kit	Roche	manual	/	columns, silica	DNA	sections (1–10 µm)	xylene	Yes (56 °C)	Yes
38	High Pure RNA Paraffin Kit	Roche	manual	/	columns, silica	RNA	sections (5–10 µm)	Hemo-De or xylene	Yes (55 °C)	No
39	GenElute Mammalian Genomic DNA Miniprep Kit	Sigma Aldrich	manual	/	columns, silica	DNA	≤ 20 mg	xylene	Yes (55 °C)	No
40	CinnaPure DNA- FFPE Tissue	SinaClon BioScience	manual	/	columns, silica	DNA	5 sections (10 µM)	xylene	Ributinase (55 °C)	No
41	Invisorb Spin Tissue Mini Kit	STRATEC Molecular	manual	/	columns	DNA	NA	octane or xylene	Yes (52 °C)	No

Table 1 | Continued.

No.	Kit	Manufacturer	DNA/RNA extraction manipulation	Instrument	Type of purification	Nucleic acids	Tissue amount	Deparaffinization	Proteinase K digestion	Reverse formalin cross-linking step
42	InviTrap Spin Universal RNA Mini Kit	STRATEC Molecular	manual	/	columns	RNA	1–8 sections (10 µm)	octane or xylene	Yes (52 °C)	Yes
43	ArrayGrade FFPE RNA Isolation Kit	Sabiosciences	manual	/	columns, silica	RNA	5–6 sections (20 µm)	xylene	Yes (37 °C)	Yes
44	TaKaRa DEXPAT Easy	TaKaRa	manual	/	absorbent resin	DNA	1–3 sections (4–10 µm)	melting in TaKaRa DEXPAT Easy (resin media)	No, boiling in resin media	No
45	SurePrep FFPE RNA Purification Kit	Fisher Scientific	manual	/	columns, resin	RNA, siRNA, miRNA	5 sections (≤ 20 µM)	xylene	Yes (50 °C)	Yes
46	WaxFree DNA Extraction Kit	TrimGen Genetic diagnostics	manual	/	columns	DNA	1 section (5–20 µm)	Q-Solution	Enzyme mix (55 °C)	No
47	WaxFree RNA Extraction Kit	TrimGen Genetic diagnostics	manual	/	columns	RNA	1 section (5–20 µm)	Q-Solution	Enzyme mix (55 °C)	No
48	FFPE DNA/RNA Extraction Miniprep System	Viogene	manual	/	columns	DNA, RNA, siRNA, miRNA	≤ 60 mg	xylene	Yes (56–60 °C)	No
49	FFPE miTotal RNA Extraction Miniprep System	Viogene	manual	/	columns	RNA, siRNA, miRNA	≤ 60 mg	xylene	Yes (56–60 °C)	No
50	ZR FFPE DNA MiniPrep	ZYMO RESEARCH	manual	/	columns	DNA	1–4 sections (≤ 20 µm)	xylene	Yes (55 °C)	Yes
51	XTRAKT FFPE Kit	Stratifyer	manual	/	paramagnetic beads	DNA, RNA, miRNA	1–3 sections (≤ 10 µm)	melting in tissue lysis buffer	Yes (65 °C)	No
52	RNeasy FFPE Kit	Qiagen	manual/ automated	QIAcube	columns, silica	RNA	1–4 sections (≤ 10 µm)	organic solvents or Qiagen Deparaffinization Solution	Yes (56 °C)	Yes
53	miRNeasy FFPE	Qiagen	manual/ automated	QIAcube	columns, silica	RNA, miRNA	1–4 sections (≤ 10 µm)	organic solvents or Deparaffinization Solution	Yes (56 °C)	Yes
54	AllPrep DNA/RNA FFPE Kit	Qiagen	manual/ automated	QIAcube	columns, silica	DNA, RNA, miRNA	1–4 sections (≤ 10 µm)	organic solvents or Qiagen Deparaffinization Solution	Yes (56 °C)	Yes
55	QIAamp DNA FFPE Tissue Kit	Qiagen	manual/ automated	QIAcube	columns, silica	DNA	1–8 sections (≤ 10 µm)	xylene	Yes (56 °C)	Yes
56	GeneRead DNA FFPE Kit	Qiagen	manual/ automated	QIAcube	columns	DNA	1 section (≤ 10 µm)	organic solvents or Qiagen Deparaffinization Solution	Yes (56 °C)	Yes
57	NucleoSpin 96 DNA FFPE	Macherey-Nagel	manual/ automated	common liquid handling instruments	membrane, silica	DNA	sections (3–20 µm)	Paraffin Dissolver or xylene	Yes (56 °C)	Yes
58	NucleoMag DNA FFPE	Macherey-Nagel	manual/ automated	common liquid handling instruments, automated magnetic separators	paramagnetic beads	DNA	sections (3–20 µm)	Paraffin Dissolver or xylene	Yes (56 °C)	Yes
59	AGENCOURT FormaPure Kit	Beckman Coulter	manual/ automated	Beckman Coulter Biomek NX or FX Span-8 workstation	paramagnetic beads	DNA, RNA, miRNA	1–5 sections (≤ 10 µm)	melting in tissue lysis buffer	Yes (55 °C)	Yes

Table 1 | Continued.

No.	Kit	Manufacturer	DNA/RNA extraction manipulation	Instrument	Type of purification	Nucleic acids	Tissue amount	Deparaffinization	Proteinase K digestion	Reverse formalin cross-linking step
60	MagMAX FFPE DNA Isolation Kit	Invitrogen	manual/ automated	MagMAX Express-96 or KingFisher Flex instruments	magnetic beads	DNA	1–2 sections (10 µm)	melting in tissue lysis buffer	Yes (60 °C)	Yes
61	MagMAX FFPE Total Nucleic Acid Isolation Kit	Invitrogen	manual/ automated	MagMAX Express-96 or KingFisher Flex instruments	magnetic beads	DNA, RNA	1–2 sections (10 µm)	melting in tissue lysis buffer	Yes (60 °C)	Yes
62	AxyPrep Mag FFPE (DNA-RNA-miRNA)	Axygen Biosciences	manual/ automated	NA	magnetic beads	DNA, RNA, miRNA	1–5 sections (≤ 10 µm)	melting in tissue lysis buffer	Yes (55 °C)	Yes
63	Prepito FFPE Kit	Chemagen	automated	Chemagic Prepito-D	paramagnetic polyvinyl alcohol beads	DNA	sections (≤ 10 µm or ≤ 5 mg)	melting in tissue lysis buffer	Yes (56 °C)	No
64	MagPurix FFPE DNA Extraction Kit	ZINEXTS	automated	MagPurix 12 instrument	magnetic beads, silica	DNA	1–8 sections (10 µm)	xylene	Yes (55 °C)	No
65	Maxwell 16 FFPE Plus LEV DNA Purification Kit	Promega	automated	AS3000 Maxwell 16 MDx Instrument LEV	silica-clad paramagnetic particles	DNA	1–10 sections (5 µm)	melting in tissue lysis buffer	Yes (70 °C)	No
66	EZ1 DNA Tissue Kit	Qiagen	automated	EZ1 instrument	magnetic beads, silica	DNA	1–5 sections (10 µm)	organic solvents or Qiagen Deparaffinization Solution	Yes (56 °C)	No
67	QIAsymphony DSP DNA Kit	Qiagen	automated	QIAsymphony SP System	magnetic beads, silica	DNA	1–4 sections (10 µm)	organic solvents or Qiagen Deparaffinization Solution	Yes (56 °C)	Yes
68	MagCore Genomic DNA FFPE One-Step Kit	MagCore	fully automated	MagCore HF16 Automated DNA/RNA Purification System	magnetic beads, cellulose	DNA	1–5 sections (≤ 5 µm)	melting in tissue lysis buffer	Yes	No
69	VERSANT Tissue Preparation Reagents Kit	Siemens	fully automated	Tissue Preparation System	magnetic beads, silica	DNA, RNA	N/A	melting in tissue lysis buffer	Yes (65 °C)	No

N/A = information not available

Discussion

Our inventory identified at least 69 commercial kits specifically developed for manual, automated, or fully automated extraction of nucleic acids from FFPE tissue specimens. The majority of commercial FFPE DNA/RNA kits employ proteolytic treatment with proteinase K to release nucleic acids from FFPE tissues. Purification of DNA/RNA molecules from lysis fluid is mostly based on silica or resin adsorption technology, although alcohol precipitation and cellulose-based purification are used as well. Many of the available kits allow removal of paraffin using special solubilisators or allow melting of paraffin directly in tissue lysis buffers, which can reduce the loss of tissue during the extraction procedure. An incubation step at an elevated temperature for partial removal of formalin cross-links of the released DNA/RNA is surprisingly used in more than half of the available kits. This particular treatment generally allows the release of longer fragments of nucleic acids, which might result in better performance in downstream assays.

Sixteen identified kits allow automated, walk-away purification of DNA/RNA from lysed FFPE tissues obtained through manual external preparations, which represents a major bottleneck for these methods and also their main drawback. Only two systems—the Siemens Tissue Preparation System/VERSANT Tissue Preparation Reagents kit and the MagCore HF16 Automated DNA/RNA Purification System/MagCore Genomic DNA FFPE One-Step Kit—have an integrated paraffin-melting/tissue-lysis step and therefore allow complete automation of nucleic acid extraction from FFPE tissues.

Because the majority of FFPE DNA/RNA extraction kits were launched in the last few years, they generally lack documented performance in peer-reviewed literature. However, recent head-to-head comparison studies suggest that these kits might differ significantly in terms of DNA yield, purity, and quality (12, 18). Therefore, it seems that the transition to one of the available FFPE DNA/RNA commercial kits will not be so straightforward and will require extensive comparisons with the established lab protocol in advance. The final decision in choosing a particular kit will probably also depend on the price and required accompanying lab equipment.

Although we identified an abundance of commercial kits specifically developed for extraction of nucleic acids from FFPE tissue specimens, many researchers are still using rather old-fashioned, crude, and probably less effective in-house methods for extracting nucleic acids from FFPE. We hope that this inventory and the accompanying comprehensive list of available commercial kits will encourage researchers to strongly consider using them in diagnostic and research settings when dealing with FFPE tissue specimens, similar to what occurred during the last decade for the great majority of other clinical specimen types.

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