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High Recovery of Nucleic Acids with Superior Yield and Purity

Agencourt® FormaPure™ System

Total Nucleic Acid Extraction from FFPE Tissue Samples

The Agencourt FormaPure system provides a simple, automation-friendly process for extracting total nucleic acid from Formalin-Fixed, Paraffin-Embedded (FFPE) tissue samples. By utilizing the patented Agencourt SPRI® (Solid Phase Reversible Immobilization) paramagnetic bead-based technology, the Agencourt FormaPure system does not require vacuum filtration, centrifugation, or organic solvents such as phenol or xylene. Researchers can go from paraffin digestion to total nucleic acid extraction in about 4 hours (without setup) for 96 samples in a multi-well format utilizing the Beckman Coulter Biomek® NX Span-8 Laboratory Automation Workstation. Recoveries are consistently higher than competitive techniques.

Key Features:

- Consistent, superior yield and purity of RNA or DNA
- Same reagents used for recovery of RNA or DNA
- No centrifugation, vacuum filtration, or solvents required
- Supports automated or manual processing
- One 96-well plate processed in about 4 hours (includes paraffin digestion step)

Superior Recovery

Agencourt FormaPure yields significantly more nucleic acid than competitive approaches. The experiment listed below was performed on rat liver tissue fixed at Agencourt Bioscience to ensure consistent starting material. When compared to currently available technologies, Agencourt FormaPure produced nucleic acid yields in excess of five times that of competitive techniques (Table 1). Ct values obtained in qPCR[2] reactions indicate that samples purified by the Agencourt FormaPure system also produce more amplifiable DNA than competitor kits (Figure 1A and 1B).

Table 1

	Agencourt FormaPure	DNeasy[1]	MagneSil[1]
Concentration (ng/µL) based on standard curve	14.83 ng/µL	2.92 ng/µL	2.52 ng/µL

Average DNA concentration from processing one 10 micron rat liver FFPE sample using Agencourt FormaPure, DNeasy Tissue kit, and MagneSil Genomic Fixed Tissue System. Elution volume for all extractions was 25 µL. A real time PCR reaction volume of 2 µL was used in the amplification of the rat beta actin gene.

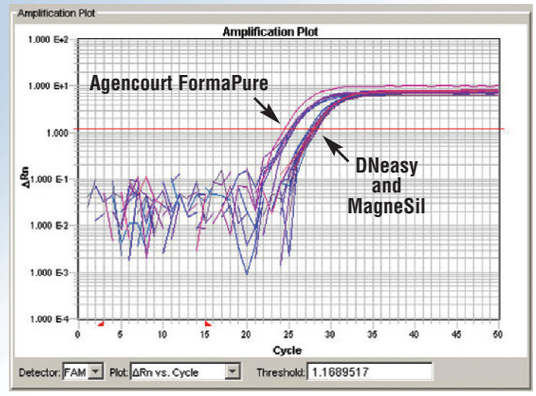

Figure 1A. *qPCR readout on an ABI Prism[1] 7900 showing DNA extracted from 10 micron FFPE tissue sections using Agencourt FormaPure, DNeasy Tissue kit and MagneSil Genomic Fixed Tissue system.*

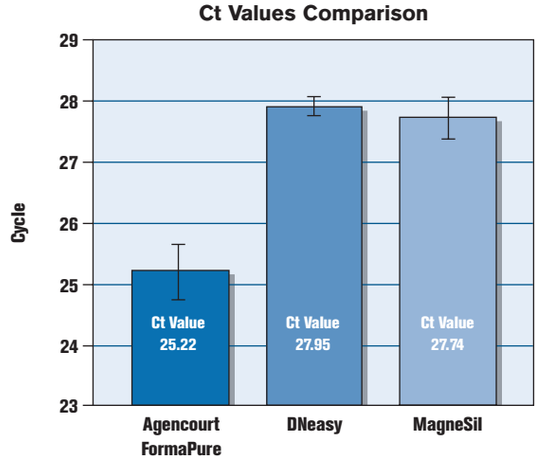

Figure 1B. *Comparison of Ct values for nucleic acid purified using the Agencourt FormaPure system, DNeasy Tissue kit and MagneSil Genomic Fixed Tissue system.*

Recovery of Fragmented Nucleic Acid

The fixation of tissue samples in formaldehyde can cause extensive crosslinking of tissue components. Quality of nucleic acid will vary depending on a variety of factors, including how the formalin-fixation process was carried out, age of sample, storage conditions, etc. As seen in Figure 2, DNA from a variety of FFPE sample types and ages was isolated using the Agencourt FormaPure process and successfully amplified by PCR.

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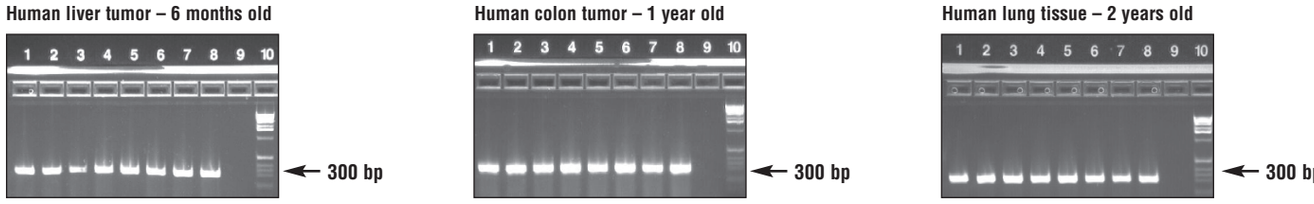

Figure 2. *Total nucleic acid was isolated from 5 micron sections for each tissue. Elution volume was 40 µL. Two (2) µL of eluted product was used in a 20 µL PCR reaction (45 cycles) to amplify a 300 bp region of the GAPDH Gene. A 10 µL sample was electrophoresed on a 2% agarose gel. Lane 9 contains the negative control. Lane 10 contains a 1 kb ladder.*

Consistent RNA Purity

The Agencourt FormaPure system provides consistent RNA yield and purity. Table 2 shows data for RNA recovered from five FFPE rat lung biological replicates which were purified using Agencourt FormaPure. Consistent ratios ranging from 1.79 to 1.83 for 260/280 and 1.06 to 1.21 for 260/230 are observed. Agilent[1] bioanalyzer traces from the same samples show consistent RNA purity and fragment recovery (Figure 3), with 18s and 28s bands clearly observed.

Table 2

Rat Lung Replicates	OD 260/280	OD 260/230	RNA Concentration (ng/µL)	Volume (µL)	Amount (µg)
1	1.79	1.21	288.99	35.00	10.11
2	1.83	1.14	439.80	35.00	15.39
3	1.79	1.07	538.57	35.00	18.85
4	1.80	1.10	629.80	35.00	22.04
5	1.80	1.06	513.13	35.00	17.96

Beta site data for RNA purified from FFPE rat lung tissues using Agencourt FormaPure. One 10 micron section was used for each replicate.

Summary

The Agencourt FormaPure system can extract both DNA and RNA and offers superior yield and purity in an automation-friendly process. This technology can purify nucleic acids from a wide variety of tissue types for utilization in techniques such as SNP genotyping, gene expression, and DNA sequencing. With the utilization of paramagnetic beads, no centrifugation, vacuum filtration or chemical solvents are required. The Agencourt FormaPure system, along with the Beckman Coulter Biomek® NX Span-8 Laboratory Automation Workstation, helps to answer today's most comprehensive biological questions.

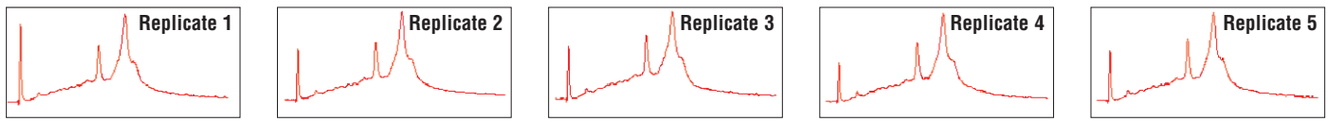

Figure 3. *Agilent bioanalyzer traces of RNA prepared from several FFPE rat lung tissues using the Agencourt FormaPure system. All samples were treated with DNAse I.*

Ordering Information

For more information, please visit our website at **www.agencourt.com** or contact your local sales representative.

Product	Size	Product #
Agencourt FormaPure Kit - Small	50 preps	A33341
Agencourt FormaPure Kit - Medium	96 preps	A33342
Agencourt FormaPure Kit - Large	384 preps	A33343

Related Products	Size	Product #
Agencourt RNAdvance™ Tissue Kit - Medium	96 preps	A32649
Biomek NX Span-8 Laboratory Automation Workstation		989884

[1] 1 All trademarks are property of their respective owners.
[2] The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffman-La Roche, Ltd.

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