

Quantitating DNA from FFPE Tissue

Superior Sensitivity of the QuantiFluor™ dsDNA System Versus the NanoDrop® Spectrophotometer

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Accurate quantitation of DNA concentration is critical for many applications. Traditional spectrophotometric assays have a practical lower limit of detection at $\sim 1\mu\text{g/ml}$; however, many isolated DNA samples have concentrations well below this level. The QuantiFluor™ dsDNA System is a fast, easy and sensitive method for determining low DNA concentrations. In this article, we isolated dsDNA from formalin-fixed paraffin-embedded (FFPE) tissue samples with the Maxwell® 16 FFPE Tissue LEV DNA Purification Kit and quantitated DNA concentrations using both the QuantiFluor™ dsDNA System and NanoDrop spectrophotometer. The NanoDrop® spectrophotometer was only able to determine concentrations for samples above $2\text{ng}/\mu\text{l}$, but the QuantiFluor™ System accurately determined concentrations for all samples.

The QuantiFluor™ dsDNA System contains a new fluorescent DNA-binding dye for quantitating small amounts of dsDNA.

Introduction

Formalin fixation and paraffin embedding (FFPE) is the most common method used worldwide for tissue storage. The Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (Cat.# AS1130) is specifically designed for optimal purification of DNA from one to ten ($5\mu\text{M}$) sections of FFPE tissue samples. To help minimize PCR inhibitor carryover, small amounts of the DNA-binding paramagnetic particles are used, and as a result the DNA-binding capacity of the system is limited to a few hundred nanograms of pure DNA suitable for amplification applications including qPCR. When working with such low levels of DNA, an accurate and sensitive method of determining DNA concentration is required. Traditional spectrophotometric assays for determining DNA concentrations such as the Nanodrop spectrophotometer require only a couple of microliters of sample to quickly determine a DNA concentration; however, the lower range of DNA concentration detectable by these instruments is only $2\mu\text{g/ml}$. Many FFPE tissue samples will produce lower concentrations of DNA than the NanoDrop instrument can accurately calculate.

The QuantiFluor™ dsDNA System^(a) (Cat.# E2670) contains a new fluorescent DNA-binding dye that enables the sensitive quantitation of small amounts of double-stranded DNA (dsDNA). This dye shows minimal binding to single-stranded DNA (ssDNA) and RNA, thus allowing specific quantitation of double-stranded DNA. This assay requires a fluorometer with a Blue optical channel ($504\text{nm}_{\text{Ex}}/531\text{nm}_{\text{Em}}$). The dye itself has little fluorescence when excited at 504nm .

Results and Discussion

Eight FFPE tissue samples, each composed of four (5 micron) sections, were purified using the Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (Cat.# AS1130). The samples were from six different tissue types: kidney, liver, spleen, heart, brain and intestinal tumor. The standard protocol was followed (TB382).

QuantiFluor™ dsDNA Analysis

The QuantiFluor™ dsDNA System is used to determine unknown DNA concentrations using a standard curve for comparison. Information on creating a standard curve and using the GloMax® Multi+ Detection System with Instinct™ Software can be found in reference 1. For this study, a five-point standard curve was created using human genomic DNA (Cat.# G3041) at final concentrations of 1, 0.1, 0.01, 0.001 and 0.0005µg/ml. One hundred microliters of each standard concentration along with a blank was added to a black 96-well plate in triplicate.

The samples were prepared as follows: the kidney, liver, spleen and brain samples were diluted 1/100 (a 1/200 final dilution after the QuantiFluor™ dsDNA Dye was added). For each of these samples, 1µl was used. The heart and three intestinal tumor samples were diluted 1/50 (a 1/100 final dilution after the QuantiFluor™ dsDNA Dye was added). For each of these samples, 2µl was used. One hundred microliters of a 1X dilution of the QuantiFluor™ dsDNA Dye was added to all the samples, standards and blanks. Following a five-minute incubation at room temperature, the samples were read and analyzed on a GloMax® Multi+ with Instinct Software. The resulting curve had a R² value of 1.0, signifying a linear fit to the curve.

The software automatically uses this resulting linear equation to fit the average of the unknown samples and determine the concentration. The resulting concentrations have low CVs and standard deviations, and all fit within the linear curve (Figure 1). The most concentrated tissue types were kidney, liver, spleen and brain, with concentrations around 5µg/ml. The remaining tissue types (heart and the intestinal tumor samples) had concentrations around 0.5µg/ml.

NanoDrop Analysis

Two microliters of the same purified DNA samples was added to the NanoDrop ND-1000 Spectrophotometer for analysis. Purity measurements (A_{260}/A_{280} and A_{260}/A_{230}) were determined simultaneously. Only the kidney, liver, spleen and brain samples had concentrations in the detectable range of the NanoDrop Spectrophotometer (Figure 2). The heart and intestinal tumor DNA samples were below the detection limit.

Comparisons

Both assays determined the concentrations of four of the eight samples with very similar results (Figure 2). A similar amount of sample was required for both assays; the NanoDrop used 2µl of sample, and the QuantiFluor™ System used 1ul for high-concentration samples or 2µl for low-concentration samples per read. The major difference

Plate map									
Step1									
Curve fit Model		Linear Fit		Equation		y = A*x + B			
Units		ug ml-1		A		78046.37663			
R2		0.99999341		B		-34.4681574			
Standard Error		0.01229415							
Wells	Label	Averaged Raw Data	Standard Concentration	Calculated Concentration	Stdev of average	CV%	Signal:Blank		
A1 B1 C1	blank	394.0463	n/a	n/a	n/a	n/a	n/a	n/a	
A2 B2 C2	S01	78420.87	1	n/a	0	0.00%	199.0143		
A3 B3 C3	S02	8010.124	0.1	n/a	0	0.00%	20.32787		
A4 B4 C4	S03	1183.08	0.01	n/a	0	0.00%	3.002388		
A5 B5 C5	S04	475.629	0.001	n/a	0	0.00%	1.207038		
A6 B6 C6	S05	456.74	0.0005	n/a	0	0.00%	1.159102		
A7 B7 C7	Kidney	2684.14	n/a	5.956873	0.102198578	1.72%	6.811737		
A8 B8 C8	Liver	2734.74	n/a	6.08654	0.119500384	1.96%	6.940148		
A9 B9 C9	Spleen	3595.66	n/a	8.292715	0.085057572	1.03%	9.124967		
A10 B10 C10	Heart	1391.493	n/a	1.322182	0.017505869	1.32%	3.531293		
A11 B11 C11	Brain	1864.36	n/a	3.856122	0.038740363	1.00%	4.731321		
A12 B12 C12	Int. tumor 1	841.052	n/a	0.6169073	0.001337619	0.22%	2.134399		
D1 E1 F1	Int. tumor 2	1159.19	n/a	1.024534	0.022036072	2.15%	2.94176		
D2 E2 F2	Int. tumor 3	784.0173	n/a	0.5438294	0.006588554	1.21%	1.989658		

Figure 1. Data calculated by Instinct™ Software. DNA purified from FFPE samples was quantitated using the QuantiFluor™ dsDNA System. The standard curve gave a R² value of 1.0, and DNA concentrations were determined for all tissue types using this derived linear equation.

Sample	NanoDrop® ND-1000 Spectrophotometer			QuantiFluor™ dsDNA System		
	µg/ml	260/280	260/230	µg/ml	Std. Dev.	CV (%)
Kidney	7.02	1.77	1.69	5.96	0.102	1.72
Liver	8.65	1.76	1.77	6.09	0.120	1.96
Spleen	9.86	1.65	1.17	8.29	0.085	1.03
Heart	N/D	—	—	1.32	0.018	1.32
Brain	4.26	1.72	1.07	3.86	0.039	1.00
Int. tumor 1	N/D	—	—	0.617	0.001	0.22
Int. tumor 2	N/D	—	—	1.02	0.022	2.15
Int. tumor 3	N/D	—	—	0.544	0.007	1.21

Figure 2. Chart comparing DNA concentrations determined by a NanoDrop® ND-1000 Spectrophotometer and the QuantiFluor™ dsDNA System.

between the assays was sensitivity. The NanoDrop® Instrument is sensitive to 2µg/ml, whereas the lower limit of the QuantiFluor™ System is 100pg/ml, or 20,000 times more sensitive. With FFPE sample types that produce more dilute samples, we were able to determine accurate DNA concentrations using the QuantiFluor™ dsDNA System but not with the NanoDrop® Instrument.

Summary

DNA was purified from various FFPE tissue sections using the Maxwell® 16 FFPE Tissue LEV DNA Purification Kit and quantitated using the QuantiFluor™ dsDNA System on the GloMax® Multi+ Instrument. The results were compared to the NanoDrop® ND-1000 spectrophotometer. The QuantiFluor™ dsDNA System accurately measured the concentration of all samples, whereas the NanoDrop® instrument failed at all but the most concentrated DNAs. The sensitivity of the QuantiFluor™ dsDNA System makes it an ideal choice for measuring the concentration of DNA isolated from trace samples like FFPE tissue.

References

- Hook, B. and Schagat, T. (2011) Application Note: *Using the QuantiFluor™ dsDNA System and the GloMax® Multi+ Fluorometer with Instinct™ Software.* #AN181. Available at: www.fluorometer.com/dyes/

Ordering Information

Product	Size	Cat.#
QuantiFluor™ dsDNA System	200 reactions	E2670
Maxwell® 16 FFPE Tissue LEV DNA Purification Ki	48 preps	AS1130
GloMax®-Multi+ Detection System with Instinct™ Software: Base Instrument with Shaking	1 each	E8032
GloMax®-Multi+ Fluorescence Module	1 each	E8051

^(a)Patent Pending.

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