Quantitating DNA from FFPE Samples Using the QuantiFluor® ONE dsDNA System and the Quantus™ Fluorometer

Promega Corporation

Materials Required

- QuantiFluor® ONE dsDNA System (Cat.# E4870, E4871)
- Quantus™ Fluorometer (Cat.# E6150)
- 0.5ml PCR Tubes (Cat.# E4941, E4942)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *Quantus™ Fluorometer Operating Manual* #TM396 and *QuantiFluor® ONE dsDNA System Technical Manual* #TM405 are available at: **www.promega.com/protocols/**

Formalin-fixed, paraffin-embedded (FFPE) tissues are valuable samples that are typically prepared from biopsies for histological examination. Because of its utility in preserving and storing tumor samples, FFPE is a useful and accessible tissue source for oncology researchers and clinicians. Many labs purify DNA from FFPE tissue for use in downstream analyses such as qPCR, multiplex PCR, microsatellite instability (MSI) analysis, agarose gel electrophoresis and next gen sequencing, among others. Isolating DNA from FFPE tissues is made simple with the Maxwell® Instrument and associated FFPE tissue DNA purification kits, which are optimized to provide the best combination of speed, purity and yield for nucleic acid purification. Accurate quantitation of the extracted DNA is critical for many downstream applications. Because many FFPE tissue sections are small and highly degraded, isolated DNA samples have concentrations well below the 2µg/ml detection limit of traditional spectrophotometric assays. Traditional spectrophotometric methods cannot provide this necessary sensitivity. In addition, they overestimate the actual concentration of DNA, causing misleading quantitation results, and thus nonoptimal downstream application results.

Real-time quantitative polymerase chain reaction (qPCR) is regarded as the 'gold standard' in the quantitative analysis of nucleic acids such as DNA. While qPCR assays are both reliable and sensitive, they require costly instruments, reagents and time-intensive manual labor. Alternatively, fluorescent binding dyes, such as QuantiFluor® Dye Systems, may be the most comparable option to qPCR as they provide the necessary sensitivity and specificity for low concentration samples, yet are quick, easy and much less expensive.

The Quantus™ Fluorometer and the QuantiFluor® ONE dsDNA System provide a rapid and sensitive method for determining DNA sample concentrations as low as 0.2ng/µl in a simple add-and-read format with no dilutions and minimal pipetting. The dye shows minimal binding to single-stranded DNA (ssDNA) and RNA, allowing specific quantitation of dsDNA. This Application Note describes the protocol for using the QuantiFluor® ONE dsDNA System with the Quantus™ Fluorometer to measure DNA purified from FFPE tissue using the Maxwell® CSC Instrument.

Conclusion

Quantitating DNA after purification is a valuable quality control measure before starting costly, time-intensive downstream analyses. qPCR is often not practical as an added step in the fast-paced workflow of an oncology laboratory. Accurate and sensitive quantitation is especially pertinent to FFPE tissues that are highly degraded and can vary widely across individual samples. In this example, NanoDrop® 2000 spectrophotometric results gave an overestimation of the actual DNA concentration by several hundredfold. The QuantiFluor® Dye with Quantus™ Fluorometer method provided quantitation results very similar to qPCR, therefore providing more accurate results.

Following DNA isolation on the Maxwell® CSC Instrument, QuantiFluor® ONE dsDNA Dye and Quantus™ Fluorometer offer a valuable quantitation step to your workflow with detection sensitivity that is comparable to qPCR yet with the simplicity and speed of an add-and-read format.

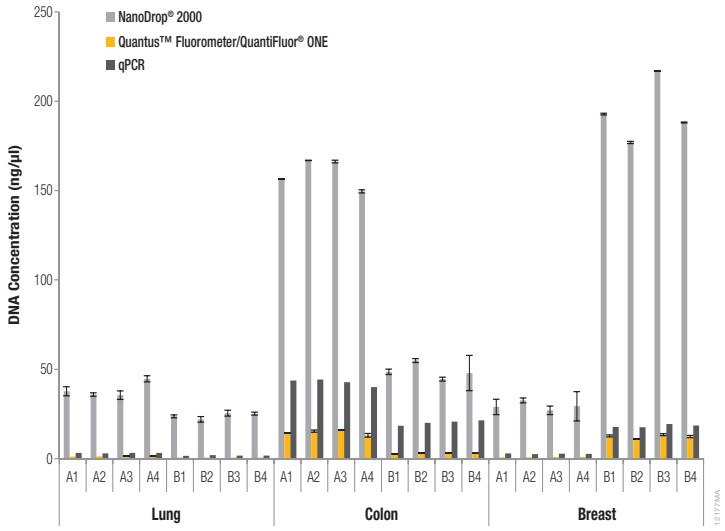

Figure 1. DNA was purified from lung, colon and breast FFPE tissue curls and quantitated using three common methods. Purification was performed with the Maxwell® CSC DNA FFPE Kit on the Maxwell® CSC Instrument. Four FFPE curls were sampled from two separate tissue blocks for all three tissue types. DNA was quantitated using the NanoDrop® 2000, Quantus™ Fluorometer with QuantiFluor® ONE dsDNA Dye, or qPCR amplification.

For the complete protocol, see the *QuantiFluor®* ONE *dsDNA System Technical Manual* #TM406.

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