Measure femtogram quantities of dsDNA

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- Nucleic acid quantification was performed on a BMG LABTECH microplate reader in fluorescence mode
- Invitrogen’s PicoGreen® assay was utilized to quantify dsDNA in 384-well HTS format
- Only 10 μL of sample is needed minimizing raw sample and waste

Introduction

In the modern molecular biology laboratory, DNA samples are a precious commodity, where only small amounts can be sacrificed for quality control analysis, preserving the majority for downstream applications such as Next Generation Sequencing. These samples often contain very small amounts of DNA and so a technique has been developed to measure DNA to femtogram levels using low volumes (10 μL), utilising a high throughput well-plate format.

With the Quant-iT PicoGreen reagent Invitrogen provides a solution for measuring low concentrations of DNA. This, together with Cambridge Biosciences AccuBlue enhancer, makes it a highly sensitive fluorescence assay for dsDNA detection.

This application note will investigate the use of very low volume DNA samples in a 384 well plate format on the BMG LABTECH filter-based microplate reader using the PicoGreen reagent in combination with Cambridge Biosciences AccuBlue enhancer.

Materials & Methods

All materials were obtained through normal distribution channels from the manufacturers stated.

- Lambda DNA, Invitrogen
- Quant-iT PicoGreen dsDNA reagent, Invitrogen
- 1x TE buffer, Promega
- AccuBlue High Sensitivity Enhancer 100x, Cambridge Bioscience
- black 384-well Optiplate-F, PerkinElmer
- Microplate Reader, BMG LABTECH
- Consumables such as pipette tips and microcentrifuge tubes were used as required from various sources.

Preparation of the Picogreen working solution

The Picogreen reagent and the AccuBlue Enhancer are diluted in TE buffer to give a final dilution of 1:100 (Picogreen) and 1:10 (AccuBlue Enhancer). For example a 500 μL final volume of working solution contains 5 μL Picogreen reagent and 50 μL AccuBlue enhancer.

Preparation of the dsDNA dilution series

A 2fold serial dilution of the stock lambda DNA solution yielded eight concentrations from 100 pg/µL to 0.78 pg/µL in 1xTE.

Results & Discussion

Figure 2 shows the dsDNA standard curve over the whole concentration range.
The standard curve was linear over the whole range from 0.39 pg/μL to 50 pg/μL. Linear regression of the data yielded an $R^2$ value of 0.9998. Standard error bars between triplicate samples are shown. After looking in more detail at the lower concentration range (Fig. 3), it can be seen that the calibration curve shows further linearity even into the femtogram per μL level.

**Fig. 3:** dsDNA standard curve using DNA concentrations from 0.39-12.5 pg/μL.

**Conclusion**

The results presented here show that using the microplate reader from BMG LABTECH it is possible to reduce the volume of the sample to 10 μL and run the tests using a 384 well plate format. The method minimises waste, typically less than 5% of sample volume, with a fast, easy and accurate microvolume nuclear acid quantitation. This technique is used routinely in our laboratory.

*The PHERAstar FSX is the newest PHERAstar reader.*