Fluorescent quantification of low amounts of dsDNA using AccuBlue™ NextGen from Biotium

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- **CLARIOstar® is able to perform sensitive detection of dsDNA ranging from 1 to 3000 pg in 96-well plates**
- **Detection in 384-well plates provides possibility conserve precious samples with excellent detection sensitivity**

Introduction

Sensitive quantification of DNA in a sample is of vital importance to applications such as forensic DNA analysis where low amounts of DNA may be present. Similarly, accurate DNA quantification is paramount for Next Generation Sequencing (NGS), where precise amounts of DNA are required to enable this powerful tool in the investigation of genetic phenomena involved in mutations that lead to cancer or disease. Fluorescent quantification methods have proven accuracy and excellent sensitivity. An analysis of cyanine dyes indicated that AccuBlue™ NextGen from Biotium exhibits the best sensitivity available[1].

Here we show the performance of DNA quantification using AccuBlue™ NextGen reagent with detection by the CLARIOstar microplate reader. The results indicate that this combination of reader and reagents provides excellent sensitivity that extends to detection in 384-well plates.

Assay Principle

Detection of low concentrations of DNA is achieved with AccuBlue™ NextGen by using a dye with low intrinsic fluorescence in the absence of nucleic acids but a large fluorescence enhancement in the presence of DNA. In addition, the use of a patented enhancer technology to further suppress background fluorescence results in an even lower limit of detection (Figure 1).

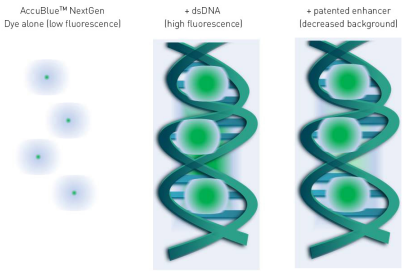

Fig. 1: AccuBlue™ NextGen dsDNA detection principle.

Materials & Methods

- AccuBlue™ NextGen dsDNA Quantification Kit (Biotium #31060)
- CLARIOstar microplate Reader (BMG LABTECH)
- Black, 96-well plates (Costar)
- Black, 384-well plates (Corning)

Standard curves were prepared using 1X AccuBlue™ NextGen Buffer and High Sensitivity dsDNA Standard (10 ng/µl), supplied with the kit. For tests using 96-well plates dsDNA dilutions were prepared ranging from 3000 to 1 pg. To show assay performance in 384-well plates dsDNA dilutions ranged from 750 to 0.25 pg.
Assays using 96 well plates were performed as described in the instructions from Biotium.

Assays using 384-well plates were performed as described with the following modifications. For the triplicate samples and buffer blank 50 µl of working solution was added to wells followed by 2.5 µl of varying concentrations of dsDNA for a standard curve or buffer for blanks.
Following incubation plates were read using CLARIOstar test protocols with the following settings:

Instrument Settings

Optic settings	Fluorescence intensity, top optic	
	Monochromator settings	Excitation: 467-15
		Dichroic: 487.2 (auto)
		Emission: 510-20
	Focus height and gain adjusted prior to reading	
General settings	Settling time: 0.2 s (96 well); 0.1 s (384 well)	
	40 flashes per well	

Results & Discussion

A previous comparison of fluorescent cyanine dyes indicated that AccuBlue™ NextGen was linear from 5 to 5000 pg[1]. Our results (Figure 2) indicate linear detection from 1 to 3000 pg in agreement with the kit literature.

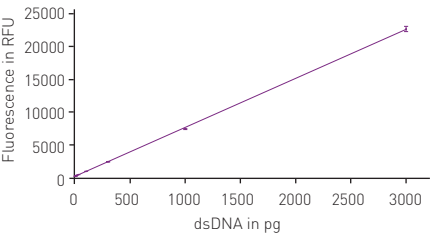

Fig. 2: Quantification of dsDNA in a 96 well plate. Plotting DNA concentration (n=3) versus relative fluorescence intensity using MARS data analysis exhibits a linear correlation (r^2=0.99983) over the indicated range.

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Figure 3 examines the same data set presented in Figure 2 but employs only the 6 lowest concentrations of DNA used. This provides a better appreciation of assay linearity at low concentrations.

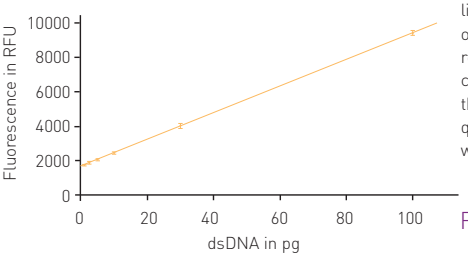

Fig. 3: RFU vs. low concentration DNA standards. Plot depicts the DNA concentration range from 1 to 100 pg (n =3). MARS data analysis shows that over this concentration range there is a strong linear correlation (r^2=0.99998).

Furthermore, previous results indicated that AccuBlue™ NextGen had a limit of detection (LOD) of 50 pg[1] using the formula:

$$LOD = x_{bl} + k\sigma_{bl}$$

Where x_{bl} is the mean of blank, σ_{bl} is the standard deviation of the blank, and k is numerical confidence factor chosen to be 3. Using the same formula we calculated the LOD to be nearly 4 pg for our 96 well assay.

Next we sought to investigate the suitability to measure this same assay in 384 well plates. Again, in this scenario, we saw that the assay was linear across the entire tested range from 750 to 0.25 pg (Figure 4). Finally we were able to calculate the LOD for this 384 well assay to be 0.309 pg.

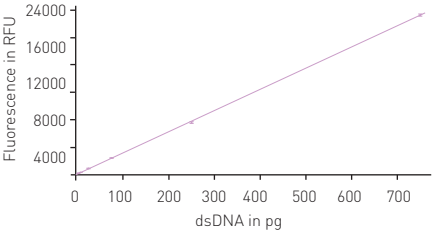

Fig. 4: Quantification of DNA in a 384 well plate assay. MARS data analysis software was used to plot the comparison of RFU to DNA concentration (n =3). The result conforms to linear fit across the entire tested range (r^2 = 0.99987).

Conclusion

The CLARIOstar plate reader has the sensitivity needed to enable optimal performance of the AccuBlue™ NextGen dsDNA Quantification Kit. We obtained excellent linearity across all DNA concentrations tested and observed LOD values that are better than previously reported. Performance extends to 384 well plates where calculated LOD is even better than in 96-well plates. Thus this combination allows for excellent sensitivity in the quantification of DNA and the ability to conserve samples when needed.

References

1. Bruijns, B.B. et al. (2016). Fluorescent cyanine dyes for the quantification of low amounts of dsDNA. *Anal. Biochem.* **511**, 74-79.

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