# Quantifying double-stranded DNA with fluorescent dyes: Hoechst 33258 measured on a FLUOstar® Omega

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- **Using Hoechst 33258 dsDNA dye for cost efficient DNA quantitation in microplate readers**
- **Specific and accurate measurement of dsDNA amount e.g. for NGS application**
- **FLUOstar® Omega provides a reliable, HTS-compatible and automatable detection platform**

## Introduction

Desoxyribonucleic acid (DNA) is the storage medium of genetic information making it a major subject of basic research and diagnostics. Nowadays, so-called next-generation sequencing (NGS) methods uncover base sequences and mutations in hours and at reasonable costs. Prerequisite for reliable sequencing data is accurate preparation and quantitation of the DNA library. Fluorescent dsDNA dyes are frequently used to quantify dsDNA as they offer high sensitivity and specificity. Various dyes are available that differ in spectral properties, DNA-binding mode, sequence preferences, detectable concentration range and costs. Hence, the dye for a specific DNA sample needs to be carefully considered.

Here, we present DNA quantification by the cost-effective Hoechst 33258 DNA binding fluorophore. Its detection on a FLUOstar® Omega resulted in high linearity and precision for measuring 11-300 ng/well dsDNA.

## Assay Principle

Hoechst 33258 is a bis-benzimide with drastically increased fluorescence intensity when bound to dsDNA (Fig. 1). The molecule binds to the minor groove of DNA and preferentially to AT-rich regions (1).

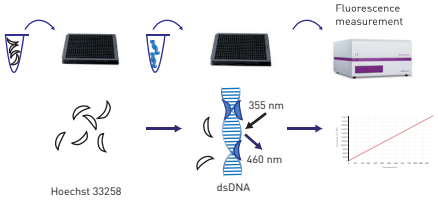


**Fig. 1:** Hoechst 33258 fluorescence is increased upon binding to dsDNA

## Materials & Methods

- black 96 well plate (Greiner #655076)
- FLUOstar® Omega, BMG LABTECH
- Hoechst 33258 (ATT Bioquest #17520, ultra pure grade, supplier: Biomol)
- Lambda phage DNA (ThermoFisher # SD0011, conc. 0.3µg/µl)

*Experimental Procedure*

Lambda phage dsDNA was dissolved in water. Hoechst 33258 was diluted to 1 or 0.1 µg/ml in TNE buffer (10 mM TRIS HCl pH 7.4; 200 mM NaCl; 1 mM EDTA) and 199 µl thereof were placed into the wells of a microplate. One microliter of DNA sample (5-300 ng/µl) was added to the dye resulting in 200 µl measurement volume per well. Standard measurements were done in triplicate. Upon thorough mixing (vortex, pipetting), fluorescence intensity was acquired using the FLUOstar Omega with the settings indicated below.

*Instrument Settings*

| | | |
|---|---|---|
| Optic settings | Fluorescence intensity, top optic | |
| | Filters | Exitation: 355 |
| | | Emission 460 |
| | Gain was adjusted prior to reading | |
| General settings | Settling time: 0.5s | |
| | 20 flashes per well | |

## Results & Discussion

The performance of the Hoechst 33258 DNA-quantitation method was tested at two Hoechst concentrations: 0.1 µg/ml and 1 µg/ml.

At the lower Hoechst concentration of 0.1 µg/ml, double-stranded lambda DNA was best quantified in a concentration of up to 100 ng per well (Fig. 2). The limit of detection (LOD) was determined using the slope of the linear regression of the standard curve and the mean of 15 blanks (no DNA): LOD = 3xSD/slope. The LOD was calculated to be 11ng dsDNA per well and correlation between fluorescence intensity and DNA concentration was highly linear as demonstrated by an $R^2$ of 0.999. The % CV between triplicates was < 4.5 %, indicating stable measurements. However, for high DNA concentrations (>100ng/µl), the fluorescent signal flattened out, most likely due to saturation of the fluorescent DNA dye.

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Keywords: dsDNA; DNA quantification; Nucleic acids

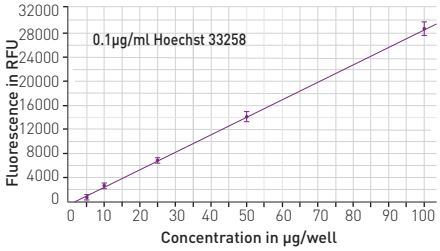


**Fig. 2:** DNA quantification with Hoechst 33258 (0.1 µg/ml). Double-stranded lambda phage DNA at indicated concentrations was mixed with the Hoechst dye and fluorescent signal was measured with the FLUOstar Omega microplate reader. Limit of detection (LOD) is 11 ng/well, $R^2$ is 0.999.

In order to extend the concentration range in which Hoechst 33258 can be used to quantify dsDNA, a higher dye concentration was tested. With a Hoechst dye concentration of 1 µg/ml the lowest detectable DNA concentration was 32.5 ng per well. The higher LOD when comparing the high fluorophore concentration to the low Hoechst concentration is explained by a higher blank that was measured with a Hoechst concentration of 1 µg/ml. However, increased Hoechst concentration enabled to measure dsDNA >100 ng/well. The measurements were linear up to 300 ng/well, the highest concentration tested.

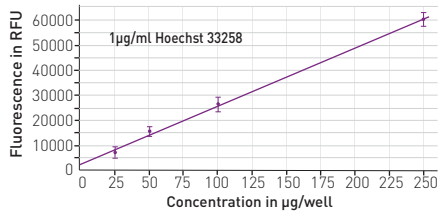


**Fig. 3:** DNA quantification with Hoechst 33258 (1 µg/ml). Double-stranded lambda-phage DNA at indicated concentrations was mixed with the Hoechst dye and fluorescent signal was measured with the FLUOstar Omega microplate reader. $R^2$ is 0.999.

## Conclusion

The use of the fluorescent Hoechst 33258 dsDNA stain together with the FLUOstar Omega multi-mode microplate reader by BMG LABTECH allows for the cost effective and specific quantification of dsDNA samples of 1-300 µg/well. The method is compatible with high throughput and can be fully automated when integrating the microplate reader into a liquid handling system.

| Hoechst concentration | Optimal range | Sample concentration range (input 1 µl) |
|---|---|---|
| 0.1 µg/ml | 11-100 ng/well<br>R2 = 0.9998 | 11-100 ng/µl |
| 1 µg/ml | >50 - min<br>300 ng/well<br>R2 = 0.9993 | >50 – 300 ng/µl |

## References

1. Pjura PE, Grzeskowiak K, Dickerson RE. (1987) Binding of Hoechst 33258 to the minor groove of B-DNA. J Mol Biol. 1987 Sep 20;197(2):257-71.

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