

# Molecular Probes® NanoOrange® Assay Performed on BMG LABTECH FLUOstar OPTIMA Microplate Reader



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Application Note 166

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- NanoOrange® Assay for protein quantitation performed on the FLUOstar OPTIMA
- High and small concentration range evaluated
- Kit can easily be adapted for high throughput measurements

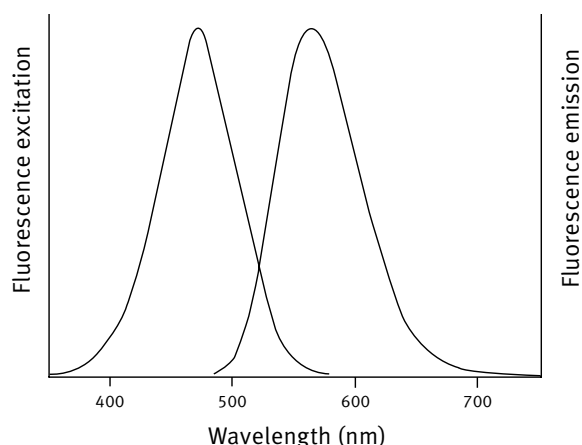
## Introduction

The field of proteomics has expanded dramatically in recent years with research on a whole host of organisms. Techniques such as two dimensional difference gel electrophoresis (2D DIGE) require the accurate quantitation of protein, as up to three labelled samples can be loaded on a single gel for comparison.

Assay methods for determining protein quantitation include absorbance at 280 nm<sup>1</sup>, the Bradford assay<sup>2</sup>, Lowry assay<sup>3</sup>, BCA method<sup>4</sup> and more sensitive assays such as Fluoroprobe® (Sigma-Aldrich) and NanoOrange® (Molecular Probes®)<sup>5</sup>.

The measurement of solution absorbance at 280 nm (A280) has problems with variability between samples and interference from nucleic acids and other contaminants. Detergents and reducing agents can cause problems with the other assays mentioned above – these agents are present in samples for 2D gel electrophoresis.

Fluorescent methods are more sensitive for quantitating proteins than absorbance methods. The NanoOrange® protein quantitation assay from Molecular Probes® is a highly sensitive assay with the useful range being between 100 ng/mL and 10 µg/mL for fluorescence-based microplate readers. The spectra of the NanoOrange® reagent is given in Figure 1.



**Fig. 1:** Excitation and emission spectra of the NanoOrange® reagent. Copyright Invitrogen Corporation. Used with permission.

The sensitivity persists over 6 hours and there is low protein-to-protein variability. Tolerance levels are as high as 100 mM for reducing agents such as DTT and β-mercaptoethanol and 0.01% for detergents such as SDS.

This application note investigates a method for using the NanoOrange® assay on the BMG LABTECH FLUOstar OPTIMA microplate reader.

## Materials and Methods

The following materials were supplied by the manufacturers as detailed:

- NanoOrange® Protein Quantitation Kit (Catalogue number N6666) (Molecular Probes®, Invitrogen, Paisley, United Kingdom)
- MJ Research PTC200 Peltier Thermal Cycler (MJ Research, now distributed by Bio-Rad, Hemel Hempstead, United Kingdom)
- FLUOstar OPTIMA Microplate Reader (Part number 413-101) (BMG LABTECH, Aylesbury, United Kingdom, Figure 2)
- Microplates, black 96 well (Catalogue number 655076) (Greiner Bio-One, Stonehouse, United Kingdom)
- General laboratory consumables included pipette tips and microcentrifuge tubes



**Fig. 2:** FLUOstar OPTIMA multidetection microplate reader

A description of the preparation for microplate assays has been noted earlier (<http://probes.invitrogen.com/media/pis/mp06666.pdf>).

### Reagents :

- NanoOrange® Protein Quantitation reagent A
- NanoOrange® Protein Quantitation diluent B
- Bovine Serum Albumin (BSA) Standard (2 mg/mL)

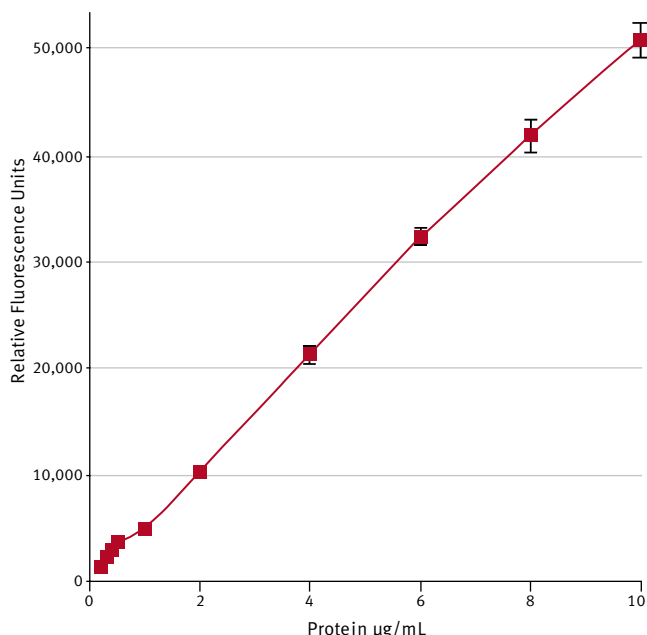
The working NanoOrange® reagent was made by diluting reagent A 500-fold in a 1:10 dilution of diluent B. A stock BSA solution at 10 µg/mL was made by diluting 1:200 in working NanoOrange® reagent. A serial dilution of the stock BSA solution was performed, yielding further concentrations ranging from 0.2 µg/mL to 10 µg/mL. The blank solution was working NanoOrange® reagent alone. Dilutions of samples were made in working NanoOrange® reagent (for example, 1:100, 1:500, 1:1000). Following denaturation of the standards and samples, 100 µL of each was placed into a microplate for measurement. The prepared 96-well plate was inserted into the instrument and read in fluorescence mode with the following parameters:

Excitation filter:	485 BP12
Emission filter:	570-10
Gain:	plate assessed, well with the highest intensity selected
Optics:	2mm combination optic
Number of cycles:	1
Number of flashes per well:	10

The data was evaluated using the BMG LABTECH FLUOstar evaluation package. The average value of the blank measurement was subtracted from the measurements and the standard curve was plotted.

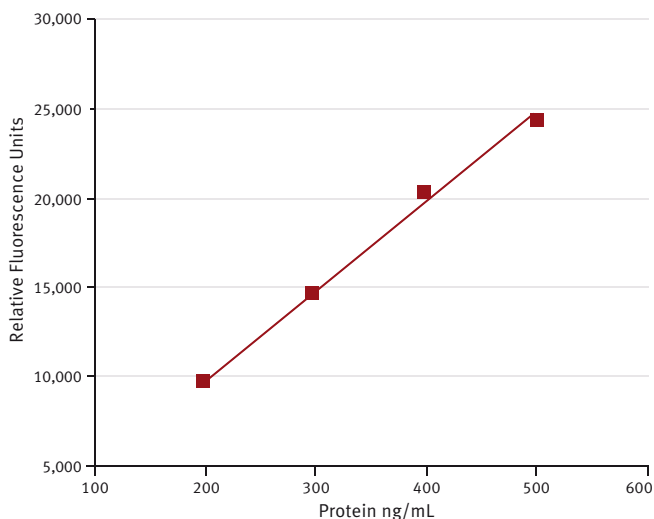
## Results and Discussion

A four parameter fit was performed on the data which yielded a very high R-value of 0.9998. (Figure 3).



**Fig.3:** BSA standard curve obtained with the NanoOrange® Protein Quantitation Kit. A BSA concentration range of 0.2-10 µg/mL is used. A gain of 1967 was optimized on the highest protein concentration.

The small concentration range was also measured separately with optimized gain on the highest protein concentration (Figure 4).



**Fig.4:** Small concentration range BSA standard curve obtained with the NanoOrange® Protein Quantitation Kit. A gain of 2721 was optimized on the highest protein concentration (12 replicates).

A linear relationship between protein concentration and fluorescence units is obtained indicating that small concentrations of protein in samples can be determined using the FLUOstar OPTIMA. Protein concentrations below 200 ng/mL can be determined with the help of the 3 mm fluorescence optic.

## Conclusion

The NanoOrange® Protein Quantitation Kit can be easily adapted for use with a fluorescent microplate reader such as the FLUOstar OPTIMA and used in a high throughput manner. NanoOrange® can also be performed on other BMG LABTECH microplate readers, including the FLUOstar and POLARstar Omegas, as well as the NOVOstar and PHERAstar.

The FLUOstar OPTIMA is a flexible multidetection microplate reader that has four different measurement modes: fluorescence intensity, time-resolved fluorescence, luminescence and absorbance. The instrument allows measurement of endpoint or slow and high kinetics at a user-defined temperature and can be easily equipped with injectors.

## References

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- [2] Bradford, M.M., (1976), A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**; 248-254.
- [3] Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J., (1951), Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**; 265-275.
- [4] Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. & Klenk, D.C., (1985), Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**; 76-85. Erratum in: *Anal. Biochem.* (1987) **163**; 279.
- [5] "NanoOrange® Protein Quantitation Kit », Product Information, Molecular Probes®, <http://probes.invitrogen.com/media/pis/mp06666.pdf>

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