A Modulus™ Microplate Fluorometer Method for

Fluorescein Measurement

INTRODUCTION

Fluorescein is a commonly used fluorophor in many research applications such as microscopy, forensics, and serology to detect latent blood stains, immunoreagent, and hybridization probe labeling, as well as reference standards for instrument calibration. The Modulus™ Microplate Fluorometer from Turner BioSystems can detect as little as 2.5 pM of fluorescein, with a linear dynamic range close to six decades.

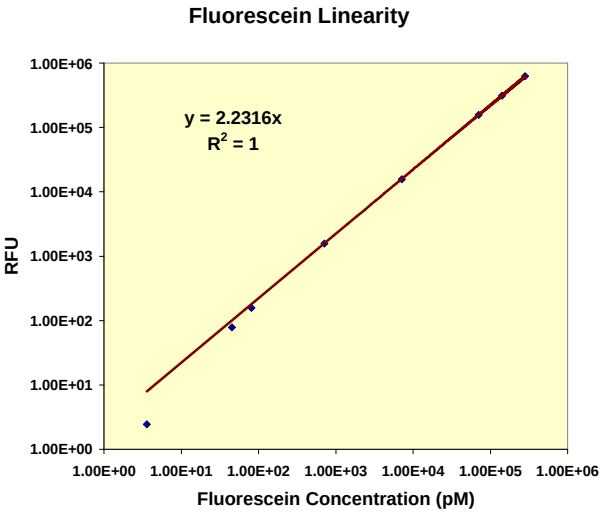

Figure 1. Broad range of fluorescein in serial dilutions, measured by the Modulus™ Microplate Fluorometer. 200-uL sample per well, n = 5.

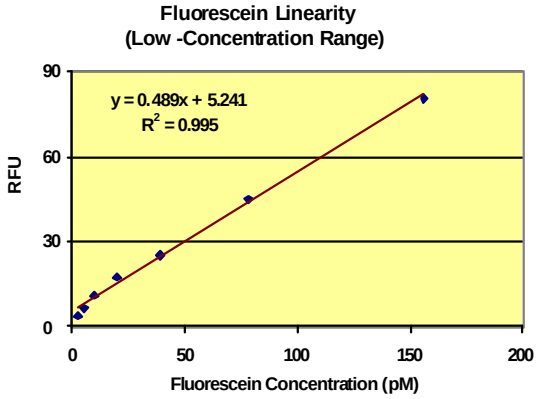

Figure 2. Low-concentration range of fluorescein measured by the Modulus™ Microplate Fluorometer. 200-uL sample per well, n=5.

MATERIALS REQUIRED

- ❑ Modulus™ Microplate Multimode Reader
- ❑ Fluorescence Optical Kit – Blue, 460/515-580 nm
- ❑ Fluorescein (Sigma, F6377)
- ❑ 96-well, black microplates (Greiner BioOne, FluoTrac 200, 655076)

EXPERIMENTAL PROTOCOL

1. Reagent Preparation Recommendation

Make a serial dilution of fluorescein in 100-mM Sodium Borate buffer. Protect the working solution from light by covering it with foil or placing it in the dark.

2. Instrument Set Up

- Insert the Blue Fluorescence Optical Kit (excitation 460 nm, emission 515-580 nm) into the Modulus™ Microplate according to the Operating Manual.
- From the Home screen, touch New Protocol to create a protocol for Fluorescein detection by following the protocol wizard.
  Enter the following: Fluorescence, Blue Optical Kit, 1 sec. integration,

and select the wells to read. Green is selected and gray is deselected. Name the protocol accordingly and select Finish.
- Refer to the on-screen Help topics, Quick Start Guide, or Operating Manual for detailed instructions.

3. Sample Analysis

- Add 200 µL of sample to each well. **Note:** If necessary, use the Plate/Well selection icon to select or deselect the wells to be read. Green is selected and gray is deselected.
- Open the instrument door by using the Door icon on the touch screen. Place the plate with A1 well at the top right corner of the microplate sample tray. Close door by using the Door icon.
- Select Start icon on the touch screen to begin a read.
- RFU values measured by the Modulus™ Microplate Fluorometer appear on the Results screen of the touch-screen display immediately after each well is measured.
- Once the measurements are complete, data can be transferred to an external computer for further data analysis in Excel by using the provided USB flash drive.
- Remove the plate after measurement completion.

RESULTS

Sensitivity: typically ~2.5 pM

Dynamic Range: linearity range was 2 pM - 625,000 pM

Minimum Detection Limit*: 0.5 fmol/200 µL (= 2.5 pM), based on 3 STDV of blank controls, n = 24

* *Data determined separately and not shown in this application note.*

CONCLUSION

The Modulus™ Microplate Fluorometer offers superior sensitivity and dynamic range for detection of fluorescein. The Modulus Microplate Fluorometer achieves its superior performance with a dedicated fluorescence detector instead of sharing the detector with other detection modes. The individual optical kit with solid-state optics and a powerful wavelength-matched LED delivers excellent sensitivity and dynamic range.

The modular approach of the Modulus Microplate Fluorometer allows for instrument capability expansion as needs in the lab change. Luminescence and/or absorbance detection modules as well as other accessories can be added after the initial purchase.

The superior performance, ease of use, and utmost flexibility of the Modulus™ Microplate make it an ideal microplate reader for today's life science laboratory.

TRADEMARK

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