Bruchez Laboratory Protocols

**MG Dye Derivatives Handling Methods**

**SUSPENSION**

**CONCENTRATION QUANTIFICATION METHOD**

**PROPER STORAGE AND USE**

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TABLE OF CONTENTS

I. Introduction
II. Dye Suspension
III. Quantifying Dye Concentration
IV. Storage and Use
V. Literature

I. INTRODUCTION

A guide for proper suspension and use of the MG derivative dyes. The appropriate suspension and concentration is critical for optimal labeling for quantification of fluorescence signal in imaging, flow cytometry, and plate reader formats.

II. DYE SUSPENSION

Dyes are shipped desiccated at .1mg/mL in plastic screw cap vials. Dissolve dye in 500 uL-1000 uL maximum of 1% Acetic acid (glacial) in ethanol*. Vortex dye solution to ensure dissolution.

*MG-B-Tau is the only dye that is recommended to be suspended in dH₂O.

III. QUANTIFYING DYE CONCENTRATION

Using a UV-VIS or similar absorbance measuring equipment:

Pipette 5 uL of dye suspension into 955 uL 1% acidic ethanol (same used for suspension) in appropriate cuvette.

Measure absorbance from 400-700 nm

The absorbance should be between .1 and 1.0 absorbance units at ~606 nm.

Example absorbance spectra is shown below in Figure 1.

Figure 1. Example Dye (MG-11p-biotin) Absorbance. A) Diluted dye in cuvette. B) Absorbance Spectra showing \( A_{606\ nm} = .59 \text{ AU} \).

Calculate the concentration using \( \varepsilon_{606} = 91 \ 600 \ M^{-1} \text{cm}^{-1} \)

Calculation Example:

91,600 M \( \text{cm}^{-1} \times \text{(Diluted Dye Concentration)} = \text{Absorbance at 606 nm.} \)

Multiply by dilution factor for original dye stock concentration.
IV. STORAGE AND USE

STORAGE
The initial quantification should last as long as no evaporation of the ethanol occurs. Re-determine the concentration of the dye at least 1x per month as evaporation is likely over this period of time. The lower the stock volume, the more likely that evaporation will occur. Storage at 4°C in screw cap vials that are tightly closed will also alleviate changes in stock concentration due to evaporation or degradation. Store in dark place.

USE
Before use the dyes are diluted into a working stock so that less than 1% of the solution is ethanol. The working stocks can be made from dH₂O, PBS, or cell culture media.
These diluted stocks should be only used for maximum a week (also stored at 4°C), freshly made stocks are always preferred for each experiment.

Labeling protocols for cell culture uses typically 100 nM dye to saturate most/all of the sites depending on expression level of the cells. Labeling occurs as quickly as 30 seconds at 500 nM, but for saturating results at lower concentrations10-30 minutes is recommended. The Kₘ for the dye/FAP complex is >1nM, needing only a small amount of dye.

For any flow cytometry experiments wash tubing thoroughly with water and ethanol in-between dye-containing samples to remove all dye from the tubing.

V. SUPPORING LITERATURE FOR ADDITIONAL INFORMATION

