Carnegie Mellon University

MBIC

Molecular Biosensor and Imaging Center

Bruchez Laboratory Protocols

MG Dye Derivatives Handling Methods

SUSPENSION

CONCENTRATION QUANTIFICATION METHOD

PROPER STORAGE AND USE

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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 dH_2O.

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.

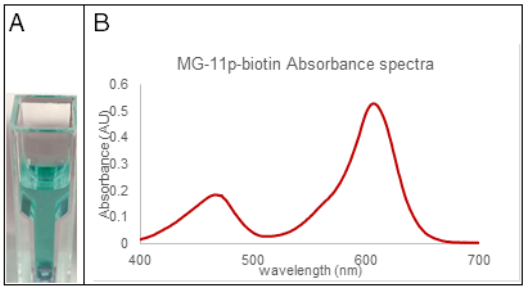

Figure 1. Example Dye (MG-11p-biotin) Absorbance. A) Diluted dye in cuvette. B) Absorbance Spectra showing $A_{606\ nm}$=.59 AU.

Calculate the concentration using ε_{606}=91 600 $M^{-1}cm^{-1}$
Calculation Example:
91,600 $M^{-1}cm^{-1}$*(Diluted Dye Concentration) = 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 dH_2O, 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 K_d 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

1. Wang, Y. *et al.* Fluorogen Activating Protein - Affibody Probes : Modular , No- wash Measurement of Epidermal Growth Factor Receptors. *Bioconjug. Chem.* (2014).
2. Yan, Q. *et al.* Near-instant surface-selective fluorogenic protein quantification using sulfonated triarylmethane dyes and fluorogen activating proteins. *Org. Biomol. Chem.* **13,** 2078–2086 (2015).
3. Szent-Gyorgyi, C. *et al.* Fluorogen-activating single-chain antibodies for imaging cell surface proteins. *Nat. Biotechnol.* **26,** 235–40 (2008).