Carnegie Mellon University

MBIC

Molecular Biosensor and Imaging Center

Bruchez Laboratory Protocols

MG Derivitive Dye Surface Labeling

DYES

LABELING PROTOCOL

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II. LIST OF SURFACE LABELING DYES

TABLE I. Commonly used dyes for surface labeling. Dye name provides link to chemical data sheet.

Dye Name	Ex/Em
MG-B-Tau	640/670
HCM-Dyedron	532/670 and 640/670

III. LABELING PROTOCOL

WORKING STOCK PREPARATION

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^oC), freshly made stocks are always preferred for each experiment.

LABELING

The dye concentration influences the incubation time needed for saturation. We suggest higher concentrations for rapid surface labeling. For fluorescence measurement experiments, maintain consistent incubation times and dye concentrations for optimal results.

If the FAP- labeled surface protein is dynamic and actively endocytosed, we recommend using a higher dye concentration and measuring fluorescence within 5

min. Alternatively, labeling for 5 min and washing excess dye is suggested for pulse-chase trafficking experiments.

TABLE II. Ideal ranges used for dyes

Dye Name	Ideal Concentration Range (nM)	Incubation Time Range (Min)
MG-B-Tau	100 - 500	15 - .5
HCM-Dyedron	100 – 1000	30 - .5

IV. 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. Naganbabu, M. *et al*. Multiexcitation fluorogenic labeling of surface, intracellular, and total protein pools in living cells. *Bioconjug. Chem.* (2016).
4. *Szent-Gyorgyi, C. et al.* Fluorogenic dendrons with multiple donor chromophores as bright genetically targeted and activated probes. *J Am Chem soc.* (2010).
5. Szent-Gyorgyi, C. *et al.* Fluorogen-activating single-chain antibodies for imaging cell surface proteins. *Nat. Biotechnol.* **26,** 235–40 (2008).