

# Amine-Reactive pHrodo<sup>™</sup> Dyes

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MAN0007048

Detailed protocol is available online at www.lifetechnologies.com/manuals.

### Storage

Store dessicated and protected from light at  $\leq$ -20°C. When stored as directed the product is stable for at least 6 months.

### Technical Specifications

- pHrodo<sup>™</sup> Green STP (P35369): Ex/Em 505/525 nm; MW = ~750
- pHrodo<sup>™</sup> Red SE (P36600): Ex/Em 560/585 nm; MW = ~650

For research use only. Not for human or animal therapeutic or diagnostic use.



## Quick Protocol

- Prepare a ~10 mM stock solution of amine-reactive pHrodo<sup>™</sup> dye by resuspending it in anhydrous DMSO (150 µL DMSO per 1 mg dye or 75 µL DMSO per 500 µg dye).
- Add the appropriate amount of pHrodo<sup>™</sup> dye to the compound in 0.1 M sodium bicarbonate buffer, pH 8.4.
  - for a purified protein, use 3–20X molar excess of pHrodo<sup>™</sup> dye
  - for purified IgG, use 10–20X molar excess of pHrodo™ dye
  - for bacteria, add pHrodo<sup>™</sup> dye to 750 µL of 20 mg/mL bacteria to obtain a final concentration of 0.5–1 M
- 3. React for 10–60 minutes at room temperature, in the dark.
- 4. Remove unreacted dye from the conjugate.
  - for proteins and IgG, use size exclusion purification or dialysis
  - for bacteria, wash 1X with HBSS, then wash 2X with methanol

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