

## pHrodo™ Red Avidin

\*Fluorogenic pH sensor\*

Catalog number P35362

**Table 1** Contents and storage

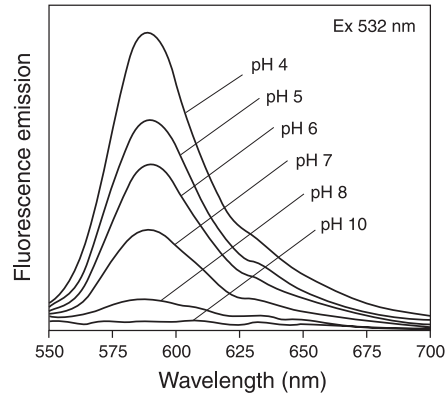
Material	Amount	Storage	Stability
pHrodo™ Red Avidin (MW = ~66 kDa) (Component A)	1 mg lyophilized product	<ul style="list-style-type: none"> <li>• ≤-20°C</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	When stored as directed this kit is stable for at least 6 months.
<b>Approximate fluorescence excitation and emission maxima:</b> 566/590 nm, diluted in 8 M Guanidine-HCl.			

## Introduction

Endocytosis or phagocytosis underlies physiological events ranging from host-pathogen response to receptor desensitization. Once internalized, material can be acidified in endosomes or lysosomes. The fluorogenic pH sensitive probe pHrodo™ Red, when conjugated to target molecules, allows direct visual monitoring of material entering these acidic compartments. At neutral and basic pH values, pHrodo™ emission is at its minimum (Figure 1, page 2), which increases with acidification by as much as 8 fold at pH 4. pHrodo™ Red is dimmed by complete media and whole blood, further improving the signal-to-noise ratio. Original pHrodo™ and pHrodo™ Red are the same molecule.

The pHrodo™ Red avidin reagent can quickly be coupled to any biotinylated reagent, including many of our own biotin conjugates of antibodies, proteins, and toxins including transferrin, EGF, and cholera toxin. Alternatively, you can create your own biotinylated target metabolically with Click-iT® chemistry to incorporate an alkyne biotin or with our biotin labeling kits (for a partial list of biotinylated probes and biotinylation kits, see page 3).

**Figure 1** The fluorescence emission spectra of pHrodo™ dye-labeled *E. coli* were measured in a series of 50 mM potassium phosphate buffers ranging in pH from 4 to 10. The *E. coli* were at a concentration of 0.1 mg/mL, and the readings were made on a Hitachi F4500 fluorometer, using an excitation wavelength of 532 nm.



## Before Starting

### Preparing Solutions

Prepare a 2 mg/mL fluorescent pHrodo™ Red Avidin conjugate working solution by dissolving the powder in 0.5 mL of PBS or other suitable buffer. Reconstituted pHrodo™ Red solutions are stable for 6–12 months if stored correctly and protected from light.

## Guidelines for Using pHrodo™ Red Avidin

- pHrodo™ Red Avidin can be used to label any biotinylated target, including secondary and primary antibodies as well as our biotinylated proteins such as EGF and transferrin. Further, you can label any target of interest that is biotinylated with our biotin labeling kits (see page 3 for a partial list of biotinylated probes and biotinylation kits).
- To complex pHrodo™ Red Avidin to any biotin containing moiety, prepare equimolar solutions of purified target material and pHrodo™ Red avidin. We recommend the addition of 1% BSA (10 mg/mL) as a stabilizer. Reconstituted solutions in BSA are stable for approximately six months at  $-20^{\circ}\text{C}$ . Protect the solutions from light, and avoid repeated freezing and thawing.
- Before use, centrifuge protein-pHrodo™ conjugate solutions briefly in a microcentrifuge and use only the supernatant for the experiment. This step eliminates any protein aggregates that may have formed in solution, thereby reducing nonspecific background staining.
- For fluorescent dye conjugates of avidins, a final concentration of 1–10  $\mu\text{g}/\text{mL}$  is usually satisfactory for most applications. Because staining protocols vary with the application, empirically determine appropriate dilutions of conjugates.
- To prevent immediate internalization, incubate the cell-pHrodo™ Red Avidin mixtures on ice for 60 minutes, wash twice at  $4^{\circ}\text{C}$  with PBS or media to remove unbound complexes, then raise the temp to  $37^{\circ}\text{C}$  and monitor. pHrodo™ conjugates can be visualized using fluorescence microscopy with a standard TRITC filter set. The dim fluorescence at pH 7.4 is normal and desired. The fluorescence will increase greatly as the pH drops. A small amount of acid solution aids visualization.
- If the basal level of pHrodo™ fluorescence is too dim to follow prior to acidification, you can prepare a control reagent. As described in references 1 and 2 (see page 3), avidin (or streptavidin) Alexa Fluor® 488 or equivalent can be coupled to your biotinylated target to visualize the material in advance of its acidification inside the cell.

## References

1. Mol BioSyst 6, 888 (2010); 2. J Virol 84, 10619 (2010); 3. J Biol Chem 286, 9373 (2011); 4. J Immunol 184, 725 (2010).

## Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
P35362	pHrodo™ Red Avidin *Fluorogenic pH sensor*	1 mg
<b>pHrodo™ Reagents</b>		
A10010	pHrodo™ <i>S. aureus</i> BioParticles® conjugate for phagocytosis	5 x 2 mg
A10025	pHrodo™ <i>E. coli</i> BioParticles® Phagocytosis Kit *for flow cytometry* *100 tests*	1 kit
A10026	pHrodo™ Phagocytosis Particle Labeling Kit *for flow cytometry* *100 tests*	1 kit
P10361	pHrodo™ dextran, 10,000 MW *for endocytosis*	0.5 mg
P35361	pHrodo™ <i>E. coli</i> BioParticles® conjugate for phagocytosis	5 x 2 mg
P35363	pHrodo™ Red Microscale Labeling Kit *for 20 to 100 µg* *3 labelings*	1 kit
P36600	pHrodo™, succinimidyl ester (pHrodo™, SE)	1 mg
<b>Biotinylated Probes and Biotinylation Kits</b>		
A10495	APEX™ Biotin-XX, Antibody Labeling Kit	1 kit
A13204	annexin V, biotin-X conjugate *100 assays*	500 µL
B1196	α-bungarotoxin, biotin-XX conjugate	500 µg
B30010	Biotin-XX Microscale Protein Labeling Kit *for 20-100 µg protein* *3 labelings*	1 kit
C34779	cholera toxin subunit B (recombinant), biotin-XX conjugate	100 µg
D20655	DSB-X™ Biotin Protein Labeling Kit *5 labelings*	1 kit
E3477	epidermal growth factor, biotin-XX conjugate (biotin EGF)	20 µg
F6347	FluoReporter® Mini-biotin-XX Protein Labeling Kit *5 labelings of 0.1-3 mg protein each*	1 kit
T23363	transferrin from human serum, biotin-XX conjugate	5 mg
Z25052	Zenon® Biotin-XX Mouse IgG1 Labeling Kit *50 labelings*	1 kit
Z25152	Zenon® Biotin-XX Mouse IgG2a Labeling Kit *50 labelings*	1 kit
Z25252	Zenon® Biotin-XX Mouse IgG2b Labeling Kit *50 labelings*	1 kit
Z25352	Zenon® Biotin-XX Rabbit IgG Labeling Kit *50 labelings*	1 kit
Z25452	Zenon® Biotin-XX Human IgG Labeling Kit *50 labelings*	1 kit
<b>ClickIT®-Biotin Metabolic Reagents</b>		
B10184	biotin azide (PEG4 carboxamide-6-azido-hexanyl biotin)	1 mg
B10185	biotin alkyne (PEG4 carboxamide-propargyl biotin)	1 mg
C10412	Click-iT® biotin DIBO alkyne *for copper free click chemistry*	1 mg

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