

Epidermal Growth Factor and Conjugates

Table 1 Contents and storage

Material	Formulation	Storage	Stability
Epidermal Growth Factor (EGF) conjugates	lyophilized powder containing EGF conjugate, lyophilized from phosphate-buffered saline (PBS), pH 7.2, containing 1% bovine serum albumin (BSA)	<ul style="list-style-type: none"> • ≤-20°C • Desiccate • Protect from light 	6 months
Approximate Fluorescence Excitation and Emission, in nm: See Table 2, page 2.			

Introduction

Epidermal growth factor (EGF) is a hormone that stimulates division of epidermal and other cells.¹⁻³ Life Technologies offers a number of EGF conjugates (Table 2, page 2), which remain fully active after conjugation and can be used to study EGF internalization.

These conjugates have proven to be quite useful for fluorometric measurements, flowcytometry, and fluorescence microscopy.⁵⁻⁷ As seen in the Table 2, page 2, fluorescence intensity of some conjugates is insensitive to pH change, but others are more sensitive to pH change. Because the pH of endosomes decreases after EGF is endocytosed, this low pH environment can be very useful for investigating the EGF endocytosis pathway. Conjugates of pHrodo™ Red and pHrodo™ Green are weakly fluorescent outside of the cells at neutral pH, but when they are internalized specifically through EGF-stimulated endocytosis, they become brightly fluorescent in acidic endosomes (Figure 1, page 2). With these reagents, minimal washing of EGF is required outside of the cells, and live dynamic measurements can be taken.

The Alexa Fluor® and Texas Red® dye-labeled EGF complexes (Table 2, page 2) are formed by biotinylating EGF at the N-terminal amino acid, and then complexing the biotinylated EGF with an Alexa Fluor® or Texas Red® streptavidin, respectively. These complexes give two to three times more signal per EGF receptor than can be obtained with a directly labeled EGF, allowing detection in cells that express low levels of the receptor.

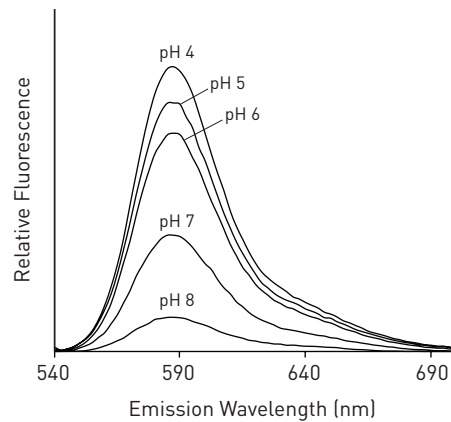
Table 2 EGF and EGF conjugates

Label	Cat. no.	Ex*	Em*	pH response	EGF type	Amount
Fluorescein	E3478	494	518	Yes	from Mouse Submaxillary Glands	20ug
Alexa Fluor® 488†	E13345	495	519	No	from Mouse Submaxillary Glands	100ug
phrodo™ Green	P35375	509	533	Yes	Human Recombinant	20ug
Alexa Fluor® 555†	E35350	555	565	No	from Mouse Submaxillary Glands	100ug
Tetramethylrhodamine	E3481	555	580	No	from Mouse Submaxillary Glands	20ug
phrodo™ Red	P35374	560	585	Yes	Human Recombinant	20ug
Texas Red®†	E3480	595	615	No	from Mouse Submaxillary Glands	100ug
Alexa Fluor® 647†	E35351	650	665	No	from Mouse Submaxillary Glands	100ug
Biotin	E3477	<300	None	No	from Mouse Submaxillary Glands	20ug
Unlabeled	E3476	NA	NA	No	from Mouse Submaxillary Glands	100ug
Unlabeled	PHG0311	NA	NA	No	Human Recombinant	100ug

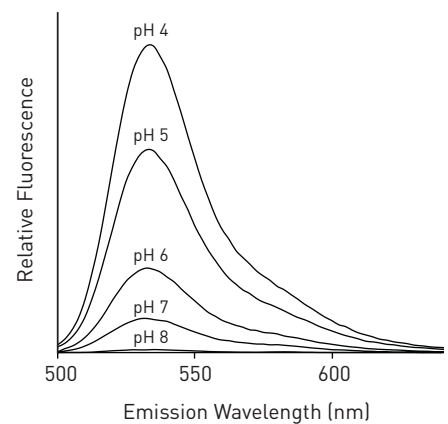
* Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm. Full spectra for these dyes are available on our website at www.lifetechnologies.com. NA = not applicable.

† A complex of biotinylated EGF and a fluorescently labeled streptavidin conjugate.

Figure 1 The fluorescence emission spectra of pHrodo™ Red and pHrodo™ Green conjugates

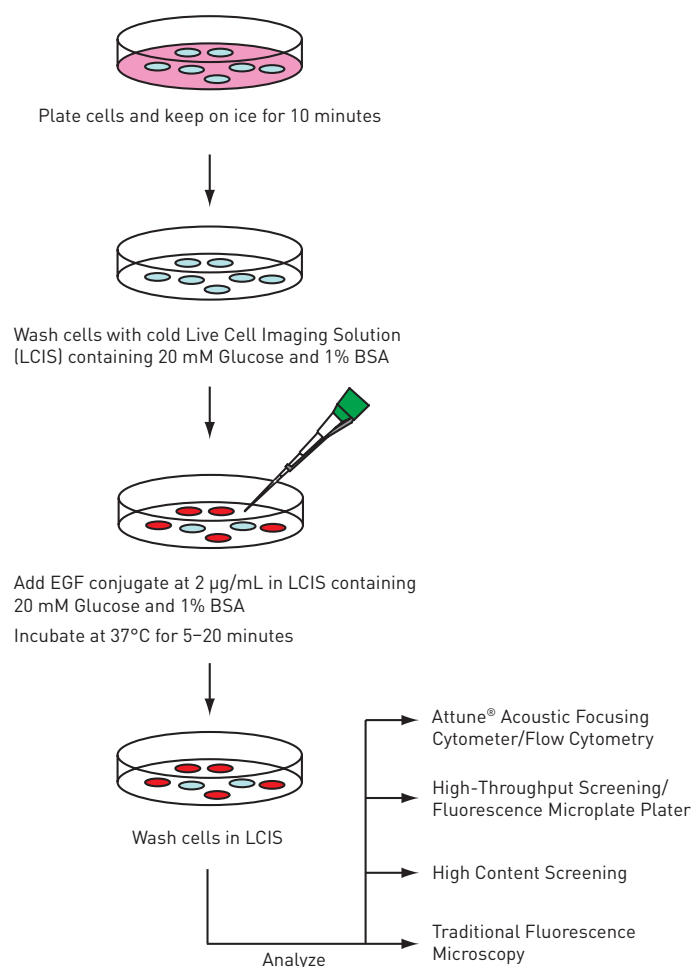


The fluorescence emission spectra of the pHrodo™ Red conjugate



The fluorescence emission spectra of the pHrodo™ Green conjugate

Figure 4 Workflow for fluorescent EGF conjugates



Materials

Contents	The products are supplied as powders lyophilized from phosphate-buffered saline (PBS), pH 7.2, containing 1% bovine serum albumin (BSA).
Storage	The lyophilized products should be stored desiccated at $\leq -20^\circ\text{C}$ until use. Allow the product to warm to room temperature before opening. Protect fluorescent conjugates and complexes from light. When stored properly, the products should be stable for at least six months.
Wash solution	Although any HEPES-based buffer at pH 7.4 can be used, we recommend using Live Cell Imaging Solution (Cat. no. A14291DJ), supplemented with 20 mM glucose and 1% BSA.
Stock solutions	These products should be reconstituted in 100 μL of deionized water, and then diluted in sufficient volume of wash solution to make a 40- $\mu\text{g}/\text{mL}$ stock solution. For longer storage, divide the solutions into single-use aliquots and freeze at $\leq -20^\circ\text{C}$. Avoid freeze-thaw cycles. Protect fluorescent conjugates and complexes from light. If aseptic techniques are not used, then 2 mM sodium azide can be added before storage.

Guidelines for Use

Application It is a good practice to centrifuge the protein conjugate solution briefly in a microcentrifuge before use; only the supernatant should then be added to the samples. This step will eliminate any protein aggregates that may have formed during storage, thereby reducing non-specific background staining.

Cells can be placed on ice for 10 minutes and cold wash solution can be used to inhibit endocytosis

Prepare EGF labeling solution by mixing an aliquot of the stock solution with the wash solution. Incubate the cells in this fresh labeling solution at 37°C for 5–30 minutes, wash, and examine. A generally suitable labeling solution is 2 µg/mL in the wash solution. This concentration can be optimized in the range of 0.5–10 µg/mL EGF for different cell lines.

Competition with unlabeled EGF may be used as a control for non-specific binding of the labeled peptide. We recommend using 20 µg/mL of unlabeled EGF, or 10 times more than labeled EGF.

When using the pHrodo™ Red or pHrodo™ Green conjugates, pH can be quantified with the Intracellular pH Calibration Buffer Kit (Cat. no. P35379).

Cells labeled with fluorescent EGF conjugates may be analyzed by fluorescence microscopy, high content screening (HCS), flow cytometry, and microplate-based fluorometry or high throughput screening (HTS).

Labeling with biotinylated EGF (Cat. no. E3477) may be followed by second-step labeling with one of the extensive selection of avidin, streptavidin, or NeutrAvidin® biotin-binding protein conjugates available from Life Technologies®. Full details of these conjugates can be obtained from our *Handbook of Fluorescent Probes and Research Products*, available at www.lifetechnologies.com or by contacting Technical Support (page 5).

References

1. J Biol Chem 265, 7709 (1990); 2. Annu Rev Biochem 56, 881 (1987); 3. Cell 61, 203 (1990); 4. EMBO J 5, 1181 (1986); 5. Proc Natl Acad Sci USA 75, 2135 (1978); 6. J Cell Biol 109, 2105 (1989); 7. Cell 96, 677 (1999).

Product List

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

Cat. no.	Product Name	Unit Size
E3476	epidermal growth factor (EGF) *from mouse submaxillary glands*	100 µg
E3477	epidermal growth factor, biotin-XX conjugate (biotin EGF)	20 µg
E3478	epidermal growth factor, fluorescein conjugate (fluorescein EGF)	20 µg
E3480	epidermal growth factor, biotinylated, complexed to Texas Red® streptavidin (Texas Red® EGF complex)	100 µg
E3481	epidermal growth factor, tetramethylrhodamine conjugate (rhodamine EGF)	20 µg
E7498	epidermal growth factor, Oregon Green® 514 conjugate (Oregon Green® 514 EGF)	20 µg
E13345	epidermal growth factor, biotinylated, complexed to Alexa Fluor® 488 streptavidin (Alexa Fluor® 488 EGF complex)	100 µg
E35350	epidermal growth factor, biotinylated, complexed to Alexa Fluor® 555 streptavidin (Alexa Fluor® 555 EGF complex)	100 µg
E35351	epidermal growth factor, biotinylated, complexed to Alexa Fluor® 647 streptavidin (Alexa Fluor® 647 EGF complex)	100 µg
P35372	pHrodo™ Red AM Intracellular pH indicator	20 µg
P35373	pHrodo™ Green AM Intracellular pH indicator	20 µg
PHG0311	Human recombinant epidermal growth factor (EGF)	100 µg
Related Products		
P35379	Intracellular pH Calibration Buffer Kit	1 kit
A14291DJ	Live Cell Imaging Solution	500 mL

Purchaser Notification

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Additional international offices are listed
at www.lifetechnologies.com

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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