Epidermal Growth Factor and Conjugates

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Desiccate
- Protect from light

Abs/Em: See Table 1

Introduction

Epidermal growth factor (EGF) is a 53–amino acid polypeptide hormone (molecular weight = 6045) that stimulates division of epidermal and other cells.[1-3] Molecular Probes' fluorescein, Oregon Green® 514, tetramethylrhodamine and biotin-XX EGF conjugates (Table 1) all contain a single fluorophore or biotin molecule on the N-terminal amino acid as confirmed by analytical HPLC. These conjugates have proven to be quite useful for fluorometric measurements, flow cytometry [4] and fluorescence microscopy.[5-7]

The Alexa Fluor® and Texas Red® dye–labeled EGF complexes (Table 1) 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, facilitating detection in cells that express low levels of the receptor.

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 ≤–20°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.

Preparation of Stock Solutions

These products should be reconstituted in 0.5 mL of deionized water to yield 40 µg/mL (for E-3477, E-3478, E-3481 and E-7498) or 200 µg/mL (for E-3476, E-3480, E-13345, E-35350 and E-35351) stock solutions in PBS, containing 1% BSA. With the addition of sodium azide to a final concentration of 2 mM, these stock solutions may be stored at 2–6°C for a few weeks. For longer storage, divide the solutions into single-use aliquots and freeze at ≤–20°C. Avoid freeze-thaw cycles. Protect fluorescent conjugates and complexes from light.

Table 1. Molecular Probes' EGF products.

Cat #	Label	Abs *	Em *
E-3478	Fluorescein	494	518
E-13345	Alexa Fluor 488 †	495	519
E-7498	Oregon Green 514	511	530
E-35350	Alexa Fluor 555 †	555	565
E-3481	Tetramethylrhodamine	555	580
E-3480	Texas Red †	595	615
E-35351	Alexa Fluor 647 †	650	665
E-3477	Biotin	<300	None
E-3476	Unlabeled	NA	NA

* Absorption (Abs) and fluorescence emission (Em) maxima, in nm. † A complex of biotinylated EGF and a fluorescently labeled streptavidin conjugate.

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 experiment. This step will eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining.

Add an aliquot of EGF stock solution to the sample of interest, incubate, wash and examine. A generally suitable labeling solution for A431 cells is 0.5–10 µg/mL EGF in Dulbecco's Modified Eagle Medium (DMEM)–HEPES buffer containing 1% BSA. Other cell lines or tissue samples may require different concentrations of EGF in different buffers. Competition with unlabeled EGF (E-3476) may be used as a control for nonspecific binding of the labeled peptide.

Cells labeled with fluorescent EGF conjugates may be viewed by fluorescence microscopy using appropriate filter sets. Labeling with biotinylated EGF (E-3477) may be followed by second-step labeling with one of Molecular Probes' extensive selection of avidin, streptavidin or NeutrAvidin™ biotin-binding protein conjugates. Full details of these conjugates can be obtained from our *Handbook of Fluorescent Probes and Research Products*, at our Web site (www.probes.com) or by contacting our Technical Assistance Department.

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 Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
E-3476	epidermal growth factor (EGF) *from mouse submaxillary glands*	100 µg
E-3477	epidermal growth factor, biotin-XX conjugate (biotin EGF)	20 µg
E-13345	epidermal growth factor, biotinylated, complexed to Alexa Fluor® 488 streptavidin (Alexa Fluor® 488 EGF complex)	100 µg
E-35350	epidermal growth factor, biotinylated, complexed to Alexa Fluor® 555 streptavidin (Alexa Fluor® 555 EGF complex)	100 µg
E-35351	epidermal growth factor, biotinylated, complexed to Alexa Fluor® 647 streptavidin (Alexa Fluor® 647 EGF complex)	100 µg
E-3480	epidermal growth factor, biotinylated, complexed to Texas Red® streptavidin (Texas Red® EGF complex)	100 µg
E-3478	epidermal growth factor, fluorescein conjugate (fluorescein EGF)	20 µg
E-7498	epidermal growth factor, Oregon Green® 514 conjugate (Oregon Green® 514 EGF)	20 µg
E-3481	epidermal growth factor, tetramethylrhodamine conjugate (rhodamine EGF)	20 µg

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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