Epidermal Growth Factor and Conjugates

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. These conjugates have been purified by HPLC after labeling and have proven to be quite useful for fluorometric measurements, flow cytometry4 and fluorescence microscopy.5-7

The Alexa Fluor® 488 and Texas Red® EGF complexes (Table 1) are formed by biotinylating EGF at the N-terminal amino acid and then complexing the biotinylated EGF with Alexa Fluor 488 or Texas Red streptavidin, respectively. These complexes give two to three times more signal per EGF receptor than can be obtained with the 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 product should be stored desiccated at -20°C until use. Allow the product to warm to room temperature before opening.

Preparation of Stock Solutions

These products should be reconstituted in 0.5 mL distilled water to yield a 40 µg/mL (for E-3477, E-3478, E-3481 and E-7498) or 200 µg/mL (for E-3476, E-3480 and E-13345) stock solution in PBS, containing 1% BSA. With the addition of sodium azide to a final concentration of 2 mM, this stock solution may be stored at 4°C for a few weeks. For longer storage, divide solution into single-use aliquots and freeze at -20°C. PROTECT FROM LIGHT. AVOID REPEATED FREEZING AND THAWING.

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.

Table 1. Molecular Probes’ EGF products.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Label</th>
<th>Abs * (nm)</th>
<th>Em * (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-3478</td>
<td>Fluorescein</td>
<td>494</td>
<td>518</td>
</tr>
<tr>
<td>E-13345</td>
<td>Alexa Fluor® 488 †</td>
<td>495</td>
<td>519</td>
</tr>
<tr>
<td>E-7498</td>
<td>Oregon Green® 514</td>
<td>511</td>
<td>530</td>
</tr>
<tr>
<td>E-3481</td>
<td>Tetramethylrhodamine</td>
<td>555</td>
<td>580</td>
</tr>
<tr>
<td>E-3480</td>
<td>Texas Red® ‡</td>
<td>595</td>
<td>615</td>
</tr>
<tr>
<td>E-3477</td>
<td>Biotin</td>
<td>&lt;300</td>
<td>None</td>
</tr>
<tr>
<td>E-3476</td>
<td>Unlabeled</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Absorption (Abs) and fluorescence emission (Em) maxima.
† Biotinylated EGF–Alexa Fluor 488 streptavidin complex.
‡ Biotinylated EGF–Texas Red streptavidin complex.
## References


## Product List

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

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Product Name</th>
<th>Unit Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-3476</td>
<td>epidermal growth factor (EGF) &quot;from mouse submaxillary glands&quot;</td>
<td>100 µg</td>
</tr>
<tr>
<td>E-3477</td>
<td>epidermal growth factor, biotin-XX conjugate (biotin EGF)</td>
<td>20 µg</td>
</tr>
<tr>
<td>E-13345</td>
<td>epidermal growth factor, biotinylated, complexed to Alexa Fluor® 488 streptavidin (Alexa Fluor® 488 EGF complex)</td>
<td>100 µg</td>
</tr>
<tr>
<td>E-3480</td>
<td>epidermal growth factor, biotinylated, complexed to Texas Red® streptavidin (Texas Red® EGF complex)</td>
<td>100 µg</td>
</tr>
<tr>
<td>E-3478</td>
<td>epidermal growth factor, fluorescein conjugate (fluorescein EGF)</td>
<td>20 µg</td>
</tr>
<tr>
<td>E-7498</td>
<td>epidermal growth factor, Oregon Green® 514 conjugate (Oregon Green® 514 EGF)</td>
<td>20 µg</td>
</tr>
<tr>
<td>E-3481</td>
<td>epidermal growth factor, tetramethylrhodamine conjugate (rhodamine EGF)</td>
<td>20 µg</td>
</tr>
</tbody>
</table>

## 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.

Please visit our Web site — [www.probes.com](http://www.probes.com) — for the most up-to-date information.

---

**Molecular Probes, Inc.**
PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 • Fax: (541) 344-6504

**Customer Service:** 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 • Fax: (541) 344-6504 • order@probes.com

**Toll-Free Ordering for USA and Canada:**
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

**Technical Assistance:** 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 • Fax: (541) 465-4593 • tech@probes.com

---

**Molecular Probes Europe BV**
Poortgebouw, Rijnburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

**Customer Service:** 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 • Fax: +31-71-5233419
euroorder@probes.nl

**Technical Assistance:** 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 • Fax: +31-71-5241883
eurotech@probes.nl

Molecular Probes' products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Several of Molecular Probes' products and product applications are covered by U.S. and foreign patents and patents pending. Our products are not available for resale or other commercial uses without a specific agreement from Molecular Probes, Inc. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to busdev@probes.com. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2001, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.