Epidermal growth factor, human (hEGF)

lyophilized, before lyophilization filtered through 0.2 µm pore size membrane
human recombinant (E. coli)

Cat. No. 11376454001 100 µg

1. What this Product Does

Contents
100 µg white lyophilizate, before lyophilization filtered through 0.2 µm pore size membrane, recombinant from E. coli.

Storage and Stability
Stable at −15 to −25°C until the expiration date printed on the label.
It is recommended to store the reconstituted solution in aliquots at −15 to −25°C.
Repeated freezing and thawing should be avoided.

Application
Epidermal growth factor (EGF) stimulates the proliferation and differentiation of a wide variety of cells of ectodermal and mesodermal origin and is a constituent of many serum-free media formulations.

Product Characteristics

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Recombinant, human epidermal growth factor (hEGF) is produced in E. coli and purified by standard chromatographic techniques.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>Human EGF is effective on human and mouse cells.</td>
</tr>
<tr>
<td>Specific activity/EC50</td>
<td>&lt;0.5 ng/ml, at least the same specific activity (EC50) compared to the indicated standard is guaranteed.</td>
</tr>
<tr>
<td>EC50 definition/</td>
<td>The concentration of hEGF that is required to support half-maximal stimulation of cell proliferation (MTT cleavage) with AKR-2B cells.</td>
</tr>
<tr>
<td>Unit definition</td>
<td>Human, recombinant EGF has the same biological activity as compared to mouse, natural EGF (1,2).</td>
</tr>
<tr>
<td>Purity</td>
<td>Recombinant, human EGF is &gt;98% pure as determined by SDS-PAGE [endotoxin (LAL-test): &lt; 100 EU/mg].</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>6,200 Da</td>
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</tbody>
</table>

2. How this Product Works

Working concentration
Human EGF exerts its biological activity in the concentration range of 0.5 - 20 ng/ml. Recommended concentration for serum-free cell culture is 1 - 10 ng/ml.

Reconstitution
In sterile water (final concentration: 500 µg/ml), further dilution with medium or PBS (phosphate buffered saline) containing 1 mg/ml BSA (bovine serum albumin), or 1-10% serum.

3. Additional Information on this Product

Background Information
Epidermal growth factor (EGF) is a small mitogenic polypeptide which is present in many mammalian species and is distributed throughout a wide number of tissues and body fluids EGF is synthesized in the tubular cells of the submaxillary gland of the mouse, in the acinar cells of the human submaxillary gland, and in the human duodenal glands (3-6). In addition, structurally and functionally related polypeptides termed transforming growth factor-α (TGF-α) and vaccinia growth factor (VGF) have been described (4-6).

Human EGF is identical to β-urogastrone, a polypeptide which was recognized and isolated on the basis of its ability to inhibit gastric acid secretion (4-7). 37 amino acids of the 53 amino acids comprising the longer urogastrone (human EGF) and mouse EGF are common to both peptides (70% homology) and the three disulfide bonds are formed in the same relative positions. The biological activity of human EGF is similar to that of mouse EGF (2). The cellular receptor for EGF is the best understood growth factor receptor. The oncogene v-erbB codes for a product homologous to a portion of the EGF receptor in which the EGF-binding domain has been deleted. Evidence exists suggesting that this truncation of the EGF receptor may lead to constitutive activation without requirement for ligand binding (8).

EGF stimulates the proliferation and differentiation of a wide variety of cells of ectodermal and mesodermal origin (e.g. fibroblasts, glial cells, keratinocytes, epithelial cells, endothelial cells, chondrocytes) (4-6, 8).

Fig. 1: [3H]-thymidine incorporation into mouse AKR-2B fibroblasts in response to human, recombinant EGF.

[AKR-2B cells were seeded at a concentration of 5.0 x 10^4 cells/ml into 96-well cell culture plates (0.1 ml/well). The culture medium used was McCoy 5A medium containing 200 mM L-glutamine and 7.5% FCS (fetal calf serum). The cells were incubated at 37°C for 4 days. When the cells were grown to confluence the culture medium was aspirated, the cells were washed twice with PBS and fresh culture medium (0.1 ml/well) was added. The culture medium for synchronisation was MCDB-402 medium containing 0.5 mg/ml insulin. The cells were again incubated for 2 days. After this incubation period the culture medium was aspirated and fresh MCDB-402 medium (0.1 ml/well) containing 0.5 mg/ml insulin was added. After 1 h various amounts of EGF were added. After another 24 h [3H]-thymidine was added. After 6 h the amount of incorporated [3H]-thymidien was determined, see also ref. 2-3, 13.)
Primary Structure
The primary structure of recombinant, human EGF (one polypeptide chain, 54 amino acids) is identical to that of human, natural EGF (β-urogastrone) (53 amino acids) but recombinant EGF has an additional methionine at the amino-terminus (1,7, 9-12).

References
12 Kishimoto, F. et al. (1986) Gene 45, 311-316.

4. Supplementary Information
Conventions
To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

<table>
<thead>
<tr>
<th>Text Convention</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbered instructions labeled 1, 2, etc.</td>
<td>Steps in a procedure that must be performed in the order listed</td>
</tr>
<tr>
<td>Asterisk *</td>
<td>Denotes a product available from Roche Diagnostics.</td>
</tr>
</tbody>
</table>

Symbols
In this Instruction Manual, the following symbols are used to highlight important information:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>☀</td>
<td>Information Note: Additional information about the current topic or procedure.</td>
</tr>
<tr>
<td>▲</td>
<td>Important Note: Information critical to the success of the procedure or use of the product.</td>
</tr>
</tbody>
</table>

Changes to Previous Version
Editorial changes.

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