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ProductInformation

ANTI-MOUSE EPIDERMAL GROWTH FACTOR (EGF)
Developed in Rabbit

Product No. **E 2635**

Antiserum is developed in rabbit using epidermal growth factor (EGF) from mouse submaxillary glands as the immunogen. The product is provided as undiluted antiserum containing 0.1% sodium azide (see MSDS)* as a preservative.

Description

Epidermal growth factor (EGF) is one of the best known and characterized growth factors. It is a small polypeptide of 53 amino acid residues and has a molecular weight of 6045 daltons. EGF stimulates epidermal cells and a variety of other cell types to divide by binding to receptor proteins on the cell surface. Binding of EGF to the receptor stimulates a chain of events including clustering of receptor molecules, activation of the receptor's kinase activity and endocytosis of receptor molecules. The biological activity of mouse and human EGF is identical. Both mouse and human EGF bind to the same receptor sites on mouse, human, rat and chick cells. EGF is important in cell culture systems and is often an essential component in serum free or reduced serum media.

Uses

Rabbit Anti-EGF may be used to monitor EGF levels in organs, biological fluids, and cell culture media by means of radioimmunoassay (RIA), enzyme immunoassay (EIA) and immunohistological techniques. The antiserum may be used as a tool to determine purity when isolating EGF.

Working Dilution

A working dilution of 1:1,000 to 1:5,000 was determined by second antibody-polyethylene glycol (PEG) RIA. Dilute antibody in 0.01 M phosphate buffered saline containing 0.1% BSA.

Storage

Store undiluted antiserum at –20 °C in working aliquots. Repeated freezing and thawing is **not** recommended.

RIA Protocol

Reagents

(A) Standards: Prepare standard solutions using EGF from mouse submaxillary glands, receptor grade (Sigma Product No. E 1257) diluted in newborn calf serum (B) to the following concentrations: 1000, 100, 50, 20, and 5 ng/ml.

(B) Newborn Calf Serum (Sigma Product No. N 4637).

(C) Samples containing EGF may be obtained from organs, biological fluids, and cell culture media. To ensure that the samples contain approximately the same amount of protein when assayed the following dilutions should be made in PBS-BSA (E):

a. Dilute the samples 1:100 and read the absorbance at 280nm.

b. If the absorbance is greater than 2.0 OD then dilute the sample 1:5-1:10 and reread the absorbance at 280nm.

c. Further dilute samples according to following table, use second and third dilutions in RIA:

Sample OD 280 Diln	2nd Diln.	Final Work Diln	3rd Diln.	Final Work
<0.1		---- 1:100	1:3	1:300
0.1-0.3	1:3	1:300	1:3	1:900
0.3-0.5	1:6	1:600	1:3	1:1800
0.5-1.0	1:10	1:1000	1:3	1:3000
1.0-1.5	1:15	1:1500	1:3	1:4500
1.5-2.0	1:20	1:2000	1:3	1:6000

(D) PBS: 0.01 M phosphate buffered saline, pH 7.4.

(E) PBS-BSA: 0.01 M PBS containing 1.0% BSA (Sigma Product No. A 7030).

(F) PBS-NRS: 0.01 M PBS containing 2.0% normal rabbit serum (Sigma Product No. R 9133).

(G) Second Antibody: Goat Anti-Rabbit IgG (whole molecule), (Sigma Product No. R 0881). Reconstitute as recommended in PBS (D).

Reagents (cont.)

(H) EDTA Solution: 0.1M ethylenediaminetetraacetic acid (EDTA) disodium salt (Sigma Stock No. ED2SS) in distilled water. Adjust pH to 7.4.

(I) PEG Solution: 6% polyethylene glycol (PEG), approximate molecular weight 8,000 (Sigma Product No. P 2139) in PBS (D).

Procedure

1. In polypropylene test tubes add:
   0.05 ml standard (A)
   0.05 ml PBS-BSA (E) **or** 0.05 ml sample (C)
   0.5 ml newborn calf serum (B).
2. Add 0.05 ml ^{125}I radioactive tracer diluted in PBS-BSA (E) to all tubes.
3. Add 0.05 ml of diluted antiserum to all tubes.
4. Vortex tubes.
5. Incubate for 2 hours at 37 °C or for 18-20 hours at room temperature.
6. After incubation add 0.1 ml EDTA solution (H) and 0.1 ml 2.0% PBS-NRS (F).
7. Vortex the tubes.
8. Add 0.1 ml second antibody (G).
9. Add 0.5 ml PEG solution (I).
10. Vortex the tubes.
11. Incubate for 5 minutes at room temperature.
12. Centrifuge at 2000 x g for 15 minutes at 4 °C.
13. Remove supernatant from each tube and determine the amount of radioactivity present in the precipitate.

Sensitivity

Sensitivity is defined as the 90% intercept of a B/B_0 standard curve. In the above system the sensitivity has been found to be 1 ng/tube.

RIA Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using the described RIA system.
K_a = 0.75 to 1.25 x 10^{10} L/mole.

Bibliography

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2. Savage, C., et al., J. Biol. Chem., **247**, 7612 (1972).
3. Fallon, J., et al., Science, **224**, 1107 (1984).
4. Schlessinger, J., et al., CRC Critical Reviews in Biochemistry, **14**, 93 (1983).

*Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

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