Technical Bulletin

Anti-Epidermal Growth Factor Antibody, Mouse Monoclonal

Clone EGF-10, purified from hybridoma cell culture

E2520

Product Description

Monoclonal Anti-Epidermal Growth Factor (EGF) (mouse IgG1 isotype) is derived from the EGF-10 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Recominant, human EGF was used as the immunogen. The isotype is determined using ImmunoType™ Kit (Cat. No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Cat. No. ISO-2). The antibody is purified by protein A.

Monoclonal Anti-Human Epidermal Growth Factor (EGF) reacts specifically with natural and recombinant EGF. The antibody does not cross-react with mouse EGF. In immunoblotting, the product stains the EGF band (6 kDa). The antibody may be used in ELISA, dot-blot, RIA and for the immunohistochemical staining of formalin-fixed, paraffin-embedded tissue.

Monoclonal Anti-Human Epidermal Growth Factor (EGF) may be used for the localization of EGF using various immunochemical assays including ELISA, immunoblotting, dot blot immunoassay, RIA and immunohistology.

Epidermal growth factor (EGF) is a single chain polypeptide hormone (6 kDa) which was first isolated from the submandibular gland of the mouse and is a potent growth-promoting factor for a variety of tissue cells in vivo and in vitro. Human EGF (hEGF) has also been extracted from human urine and shown to be identical or closely related to β-urogastrone, a potent inhibitor of gastric acid secretion.[1] Human EGF is structurally homologous to human transforming growth factor-α (TGFα), which also exerts its actions through EGF receptors. It is homologous to a sequence contained in a 19 kDa protein of Vaccinia virus, which appears to utilize the EGF receptor to gain entry into cells.[2]

Cloning data for mouse and human EGF suggest that the 53 amino-acid EGF peptide is cleaved from a 125-130 kDa, 1217 amino-acid precursor containing an N-terminal 29 amino-acid signal sequence. Mouse and human EGF exhibit 70% similarity at the amino acid level and are highly species cross-reactive. Structures obtained for both mouse and human EGF suggest that the protein exists largely in a β-sheet conformation, with a C-terminal hairpin that may be involved in receptor binding. EGF acts through a specific cell surface receptor glycoprotein of approximately 170 kDa. EGF binding induces phosphatidylinositide hydrolysis and generation of diacylglycerol (DAG). DAG-dependent activation of protein kinase C, in turn, results in the phosphorylation of a threonine residue at position 654 of the receptor, in attenuation of the intrinsic tyrosine kinase activity, and subsequently alterations of receptor homeostasis.[3] Cellular metabolic effects of EGF include stimulation of ion fluxes, glucose transport, glycolysis and synthesis of DNA, RNA and proteins. EGF acts on a variety of tissues and binding has been demonstrated in virtually all cell types tested, with the exception of those of the hematopoietic lineage. EGF is mitogenic for a variety of epidermal and epithelial cells, including fibroblasts, glial cells, mammary epithelial cells, vascular and corneal endothelial cells, bovine granulosa, rabbit chondrocytes, HeLa and SV40-3T3 cells.[4] Human EGF is found in body fluids such as urine, milk, saliva, sweat, and seminal fluid and has its highest concentration in α-granules of blood platelets. It has a widespread distribution in human tissues and organs, including neoplasms. The biological role of EGF includes the inhibition of gastric acid secretion, support of growth and differentiation during fetal development, neuromodulation in the central nervous system and stimulation of epidermal growth and keratinization. Since EGF plays a role in the proliferation and/or differentiation of normal as well as tumor cells derived from a variety of tissues,[5] and can contribute to pathological states, an in vitro assay for its detection and quantification is desirable. Monoclonal antibody reacting specifically with EGF may be used in the determination and quantification of the molecule in many in vitro systems and in vivo human models, and for studies on structure-function relationship of this factor in binding to the receptor molecule.[6]

Reagent

The antibody is provided in 0.2 µm-filtered 0.01 M phosphate buffered saline.

Protein Concentration: 2 mg/mL by absorbance at 280 nm.

Product Profile

A working concentration of at least 0.1 µg/mL was determined by dot blot immunoassay using recombinant human EGF.

In order to obtain best results, it is recommended that each individual user determine their working dilution by titration assay.

Preparation Instructions

Dilute product to the working concentration using 0.2 µm-filtered phosphate buffered saline or Hank’s balanced salt solution. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

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4. Carpenter, G., and Cohen, S., Ann. Rev. Biochem., 48, 193 (1979).
5. Mori, M., et al., Acta Histochem. Cytochem., 22, 15 (1989).
6. Nishikawa, K., et al., Meth. Enzymol., 146, 11 (1987)

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