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Product Information

Anti-phospho-Epidermal Growth Factor Receptor pTyr^{845}
produced in rabbit, affinity isolated antibody

Catalog Number **E0907**

Product Description

Anti-phospho-Epidermal Growth Factor Receptor pTyr^{845} is produced in rabbit using as immunogen a synthetic phosphopeptide corresponding to residues surrounding Tyr^{845} of human EGF R (Gene ID: 1956). This polyclonal antibody was affinity-purified on a column derivatized with the phosphopeptide, and further purified by protein A chromatography.

Anti-phospho-Epidermal Growth Factor Receptor pTyr^{845} detects endogenous human EGF R phosphorylated at Tyr^{845}. Reactivity with other species has not been determined. The antibody may be used in various immunochemical techniques including immunoblotting, immunohistochemistry, and flow cytometry.

The epidermal growth factor (EGF) family of receptor tyrosine kinases consists of four receptors, EGFR (ErbB1), ErbB2 (neu), ErbB3, and ErbB4. Members of the EGF R family contain 3 domains: an extracellular domain that is involved in ligand binding and receptor dimerization, single transmembrane domain, and cytoplasmic domain. EGF exerts its actions by binding to the EGF receptor, a 170 kDa protein.

EGF R, also known as ErbB1, is a type transmembrane glycoprotein receptor tyrosine kinase. Upon binding of one of the EGF family ligands, EGF R can form homodimers as well as heterodimers with ErbB2, ErbB3, or ErbB4. EGF R regulates cell proliferation, differentiation, motility, and apoptosis in a wide variety of cell types.

Activation of EGF receptor results in initiation of diverse cellular pathways. In response to toxic environmental stimuli, or to EGF binding to the receptor, the EGFR forms homo- or heterodimers with other family members.[1] Each dimeric receptor complex initiates a distinct signaling pathway by recruiting different Src homology 2 (SH2) containing effector proteins.

Dimerization results in auto-phosphorylation on various residues within the cytoplasmic domain, as well as phosphorylation of intracellular substrates, initiating a downstream cascade of events. The activated EGF receptor dimer forms a complex with the adaptor protein Grb that is coupled to the guanine nucleotide releasing factor, SOS. The Grb-SOS complex can either bind directly to phosphotyrosine sites or indirectly through Shc. These protein interactions bring SOS in close proximity to Ras, allowing for Ras activation. This subsequently activates the Erk and JNK signaling pathways that in turn activate transcription factors, such as c-fos, AP-1, and ELK-1 resulting in increased gene expression and cell proliferation.[2-4]

Reagent

Supplied as approximately 50 μg (sufficient for 100 mL of blotting solution) of lyophilized product from a 0.2 μm filtered solution in phosphate buffered saline with 5% trehalose.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reconstitute in phosphate buffered saline containing 0.02% sodium azide.

Storage/Stability

Store lyophilized product at –20 °C or below. Lyophilized samples are stable for twelve months from date of receipt when stored at –20 °C or below.

Upon reconstitution, the antibody can be stored at 2-8 °C for up to one month without detectable loss of activity. For extended storage, upon reconstitiution, the solution should be frozen at –20 °C or below in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

Immunoblotting: a working concentration of 0.5 µg/mL is recommended. A431 human epidermoid carcinoma cells were either untreated or treated with 100 ng/mL EGF and harvested five minutes after stimulation. Total cell lysates were resolved by SDS-PAGE, transferred to an Immobilon-P membrane, and immunoblotting was performed with 0.5 µg/mL of the antibody and a one minute exposure to film..

Immunohistochemistry: this antibody was used at a concentration of 15 µg/mL with the appropriate secondary reagents to detect EGF R phosphorylated at Tyr^{845} in paraffin-embedded sections of human sarcoma and glioblastoma. Chromogenic detection of labeling is recommended.

Flow cytometry: the antibody should be diluted to 0.5 µg/mL and 10 µL is recommended using A431 human epidermoid carcinoma cells either untreated or treated with 100 ng/mL EGF for five minutes.

Note: For intracellular staining, cells must first be fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin in phosphate buffered saline. Dilute this antibody to 5 µg/mL and add 10 µL of the diluted solution to 1-5 x 10^5 cells in a total reaction volume not exceeding 200 µL. Following a 30 minute incubation, cells should be washed with 0.1% saponin prior to addition of a secondary developing reagent. The binding of unlabeled primary antibodies may be visualized by adding 10 µL of a 25 µg/mL solution of a secondary developing reagent such as anti-Rabbit IgG conjugated to a fluorochrome. Cells should be washed for a final time in 0.1% saponin prior to flow cytometric analysis.

Note: In order to obtain the best results in various assays, it is recommended that each individual user determine their working dilution by titration.

References

1. Wells, A., EGF receptor. *Int. J. Biochem. Cell Biol.*, **31**, 637-643 (1999).
2. Quan, X., et al., N terminus of Sos 1 Ras exchange factor: critical roles for the Dbl and plecstrin homology domains. *Mol. Cell Biol.*, **18**, 771-778 (1998).
3. Lanzetti, L., et al., The Eps8 protein coordinates EGF receptor signaling through Rac and trafficking through Rab5**.** *Nature*, **408**, 374-377 (2000).
4. Poppleton, H.M., et al., Modulation of the protein tyrosine kinase activity and autophosphorylation of the epidermal growth factor receptor by its juxtamembrane region. *Arch. Biochem. Biophys.*, **363**, 227-236 (1999).

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