ProSci INCORPORATED
www.prosci-inc.com

12170 Flint Place
Poway, CA 92064
Tel: 858-513-2638
Fax: 858-513-2692
Toll Free: 888-513-9525
techsupport@prosci-inc.com

EGFR [pY1068] Antibody

CATALOG NO.: XBP-4086

BACKGROUND:

Epidermal growth factor receptor (EGFR, a 185 kDa glycoprotein), also known as ErbB-1, is a transmembrane tyrosine kinase that regulates a variety of biological responses ranging from mitogenesis to stress signaling. The EGFR consists of a large extracellular domain, a single transmembrane domain and a cytoplasmic domain that exhibits kinase activity. Upon binding of EGF to the extracellular domain, the receptor undergoes dimerization and becomes phosphorylated on several tyrosine residues within the cytoplasmic domain. These events result in EGFR activation and increased tyrosine kinase activity toward a variety of intracellular substrates. Tyrosine 1068, within the cytoplasmic domain of the receptor, is a major autophosphorylation site that allows binding of Grb2 and activation of the Ras to Raf to ERK1&2 signaling pathway.

SPECIFICITY:

Human EGFR. Mouse and rat (100% homologous) EGFR have not been tested, but are expected to react.

SOURCE:

EGFR antibody was produced against a chemically synthesized phosphopeptide derived from the region of human EGFR that contains tyrosine 1068. The sequence is conserved in rat.

EGFR antibody was purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated epidermal growth factor receptor (EGFR). The final product is generated by affinity chromatography using an EGFR-derived peptide that is phosphorylated at tyrosine 1068.

APPLICATION:

For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. **This product is for research use only.**

STORAGE:

Store at -20ºC. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at -20ºC. For shipment or short-term storage (up to one week), 2-8ºC is sufficient.

(07-02D)

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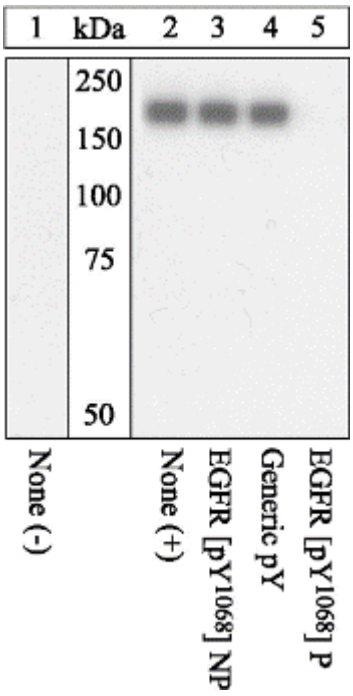

Lysates from A431 cells unstimulated (lane 1) or stimulated with 200 ng/mL EGF for 15 minutes (lanes 2-5), were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer for one hour at room temperature, and then incubated with the EGFR [pY1068] antibody for two hours at room temperature in a 1% BSA-TBST buffer, following prior incubation with: no peptide (lanes 1 and 2), the non-phosphopeptide corresponding to the phosphopeptide immunogen (lane 3), a generic phosphotyrosine-containing peptide (lane 4) or the phosphopeptide immunogen (lane 5). After washing, the membrane was incubated with goat F(ab')2 anti-rabbit IgG HRP conjugate and signals were detected using the Pierce SuperSignal(TM) method. The data show that only the phosphopeptide corresponding to EGFR [pY1068] blocks the antibody signal, demonstrating the specificity of the antibody. The data also show the induction of EGFR [pY1068] phosphorylation by the addition of EGF to this cell system.