

DESCRIPTION

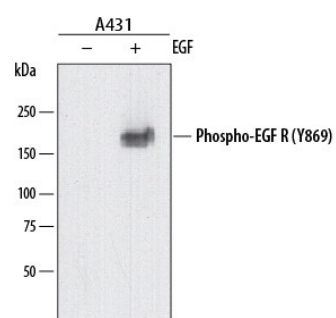
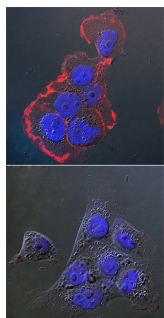
Species Reactivity	Human
Specificity	Detects human Phospho-EGFR (Y869) in ELISA.
Source	Monoclonal Rat IgG _{2B} Clone # 869111
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Phosphopeptide containing the human EGFR Y869 site
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below

DATA

<p>Western Blot</p>  <p>Detection of Human Phospho-EGFR (Y869) by Western Blot. Western blot shows lysates of A431 human epithelial carcinoma cell line untreated (-) or treated (+) with 100 ng/mL Recombinant Human EGF (Catalog # 236-EG) for 5 minutes. PVDF membrane was probed with 0.5 µg/mL of Rat Anti-Human Phospho-EGFR (Y869) Monoclonal Antibody (Catalog # MAB8130) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). A specific band was detected for Phospho-EGFR (Y869) at approximately 190 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p>  <p>Phospho-EGFR (Y869) in A431 Human Cell Line. EGFR phosphorylated at Y869 was detected in immersion fixed A431 human epithelial carcinoma cell line untreated (lower panel) or treated (upper panel) with Recombinant Human EGF (Catalog # 236-EG) using Rat Anti-Human Phospho-EGFR (Y869) Monoclonal Antibody (Catalog # MAB8130) at 25 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rat IgG Secondary Antibody (red; Catalog # NL013) and counterstained with DAPI (blue). Specific staining was localized to plasma membrane upon stimulation. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

EGF receptor, also known as ErbB1, is an approximately 160 kDa transmembrane receptor tyrosine kinase that binds multiple EGF family proteins. Ligand binding induces EGFR homodimerization or heterodimerization with ErbB2, 3, or 4 as well as activation of its kinase domain and phosphorylation within the cytoplasmic domain. Phosphorylation of Tyr869 by Src is important for full activation of the receptor. Phosphorylation of Tyr1197 by MAP kinases contributes to EGFR interaction with PIK3C2B. EGFR signaling regulates multiple biological functions including cell proliferation, differentiation, motility, and apoptosis. Three additional alternate splice forms lack the transmembrane and cytoplasmic domains. Within the ECD, human EGFR shares 88% aa sequence identity with mouse and rat EGFR.