Human Phospho-EGFR (Y845) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF3394

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human EGFR when phosphorylated at Y845.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide containing human EGFR Y845 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.

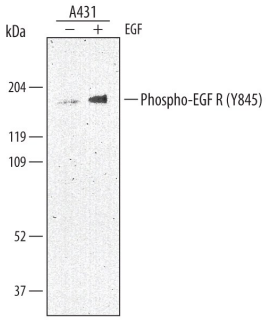
APPLICATIONS

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	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	Immersion fixed paraffin-embedded sections of human glioblastoma and sarcoma subjected to Antigen Retrieval Reagent-Basic (Catalog # CTS013)
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

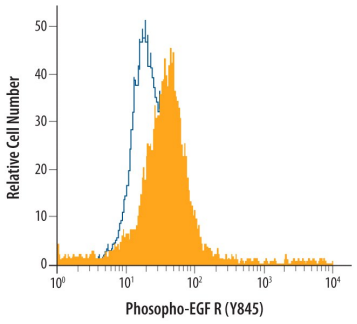
DATA

Western Blot



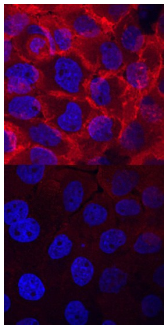
Detection of Human Phospho-EGFR (Y845) by Western Blot. Western blot shows lysates of A431 human epithelial carcinoma cell line untreated (-) or treated (+) with 100 ng/mL Recombinant Human EGF (Catalog # 236-EG) for 5 minutes. PVDF membrane was probed with 0.5 µg/mL of Rabbit Anti-Human Phospho-EGFR (Y845) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3394), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Phospho-EGFR (Y845) at approximately 175 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Intracellular Staining by Flow Cytometry



Detection of Phospho-EGFR in EGF-treated A431 Human Cell Line by Flow Cytometry. A431 human epithelial carcinoma cell line was untreated (open histogram), or treated for 5 minutes with 100 ng/mL Recombinant Human EGF (Catalog # 236-EG; filled histogram) then stained with Rabbit Anti-Human Phospho-EGFR (Y845) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3394), followed by Phycoerythrin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0110). Rabbit IgG (Catalog # AB-105-C, data not shown) was used as a control antibody. To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

Immunocytochemistry



Phospho-EGFR (Y845) in A431 Human Cell Line. EGFR phosphorylated at Y845 was detected in immersion fixed A431 human epithelial carcinoma cell line untreated (lower panel) or treated (upper panel) with pervanadate using Rabbit Anti-Human Phospho-EGFR (Y845) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3394) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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.** ● 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

Epidermal growth factor receptor (EGFR, also known as ErbB1/HER1) is the founding member of the EGFR family of receptor tyrosine kinases. Ligand binding induces receptor dimerization and autophosphorylation on multiple tyrosine residues. Phosphorylation of tyrosine 845 is associated with regulation of receptor function and tumor progression.

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